Occupational leptospirosis in New Zealand

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Abstract

Although a decreasing trend of human notifications was observed from 2001 to 2014 (Chapter 1), the incidence of human leptospirosis in New Zealand continues to be higher than in other high-income countries and affecting predominately people occupationally exposed to livestock (i.e. abattoir workers and farmers). Additionally, evidence suggests that leptospirosis may have a higher detrimental effect on production in deer compared with beef cattle or sheep. It was also observed that vaccination against *Leptospira* of not previously infected animals reduce the risk of urinary shedding of leptospires after challenge, and that there is limited evidence supporting or disproving that maternally derived antibodies interfere with the effect of vaccination when animals are vaccinated at a young age.

When sero-positivity was defined as a serum microscopic agglutination test (MAT) titre of $\geq$48, 6.6% of farmers (Chapter 2) and 5.1% of veterinarians (Chapter 3) were sero-positive to at least one of five *Leptospira* serovars (Hardjo-bovis, Pomona, Copenhageni, Ballum, Tarassovi). Veterinarians had a higher risk of being sero-positive when they slaughtered cattle or pigs at home or worked in a mixed animal practice. Assisting calving of cattle or deer, farming deer alone or in combination with cattle and/or sheep, flat terrain on farm, and abundance of wild deer on farm, increased *Leptospira* sero-positivity of farmers. Apart from vaccinating farmed livestock, increased awareness of such risk factors and the use of protective equipment may reduce the human leptospirosis incidence in these occupational groups.

Similar to earlier observations in abattoir workers, *Leptospira* sero-prevalence of farmers and veterinarians was associated with the recall of influenza-like illness of sampled individuals. Using the estimated incidence of influenza-like illness attributable to *Leptospira* infection (population attributable risk) of veterinarians (0.05%), farmers (1.3%) and abattoir workers (2.7%), we quantified the burden of human leptospirosis in terms of disability-adjusted life years (DALYs) and economic

cost of infection; the latter including the cost of vaccination, which is primarily used in dairy cattle (Chapter 4). Annual DALYs were estimated to be 0.43 per 100,000 people in New Zealand, and 16.76 per 100,000 people when only considering the occupationally-exposed population (abattoir workers, farmers, veterinarians). This ranks leptospirosis in New Zealand's high-risk population similar to worldwide estimates of DALYs for rabies and dengue. The total annual cost of leptospirosis due to human disease (i.e. treatment and absence from work); production loss in deer, beef cattle, and sheep; and the cost for vaccinating them was estimated to be NZ$25.36 million. One third of this total was attributed to vaccination of dairy cattle. The annual cost of human treatment and workplace absence due to severe and mild leptospirosis was NZ$4.49 million. Total lost production cost was NZ$11.31 million, half of which was attributable to reproductive and growth reduction in deer. No estimates are currently available from any other country for the public health burden and the overall economic loss including farmed livestock for this disease.

Since vaccination of livestock is currently regarded as the most effective means of preventing human exposure, the literature on the efficacy of *Leptospira* vaccines for preventing urinary shedding was systematically reviewed (Chapter 5). The meta-analysis of vaccination trial results, using articles with sufficiently detailed data, resulted in a pooled vaccine efficacy estimate of 82% when shedding was assessed by culture.

The findings of this thesis contribute towards a better understanding of the public health burden, economic cost, infection sources for humans, and the efficacy of vaccination for reducing the risk of *Leptospira* urinary shedding in domestic livestock.

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Declaration

This thesis was formatted as five independent research chapters suitable for journal publication. Therefore, concepts and/or methodology described in a chapter may be repeated in another chapter. My input as main author of this research was to plan and coordinate sampling of veterinarians and farmers, develop questionnaires to record relevant information, process samples in the laboratory and test them for antibodies against *Leptospira*, conduct systematic literature search, select studies for meta-analysis, analyse data, and draft manuscripts reporting findings. People that contributed substantially to the research were made co-authors as listed at the beginning of each chapter.

List of abbreviations

MUHEC: Massey University Human Ethics Committee
MAT: Microscopic Agglutination Test
OR: Odds Ratio
RR: Relative Risk
PR: Prevalence Ratio
CI: Confidence Interval
PI: Probability Interval
PAR: Population Attributable Risk
PAF: Population Attributable Fraction
LRT: Likelihood Ratio Test
REML: Restricted Maximum Likelihood
MCMC: Markov Chain Monte Carlo
DALYs: Disability-Adjusted Life Years
YLL: Years of Life Lost
YLD: Years Lost due to Disability
MDA: Maternally Derived Antibodies
IgM: Immune Globulin of class M
IgG: Immune Globulin of class G
PBMC: Proliferation of peripheral Blood Mononuclear Cells
PCR: Polymerase Chain Reaction
FA: Fluorescent Antibody
DFM: Dark Field Microscopy
ACC: Accident Compensation Corporation

List of publications

Benschop, J., C. Heuer, J. Collins-Emerson, L. Stringer, J. Sanhueza, and P. Wilson (2012). "An independent review of leptospirosis vaccination guidelines". In: *Proceedings of the Society of Dairy Cattle Veterinarians of the New Zealand Veterinary Association*. Vol. 66, pp. 2.13.1–2.13.5.

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Introduction

Leptospirosis is a re-emerging zoonosis of worldwide distribution that affects a wide range of animal species. Once an animal is infected, the bacteria colonise the kidneys and are excreted into the environment through urine, exposing other animals and humans. Signs and symptoms of leptospirosis range from a subclinical or mild influenza-like illness to a severe disease that can cause renal and hepatic damage and death. Additionally, about a third of human leptospirosis cases experience long term sequelae.

In New Zealand, after the introduction of annual leptospirosis vaccination in dairy cattle, the number of human notified cases dropped from an average of 11 per 100,000 in the 1970s to an average of 4.5 per 100,000 in the period from 1981 to 1984. In recent years (2010–2014), the average of leptospirosis notified cases was 1.9 cases per 100,000. However, this incidence of leptospirosis notifications still ranks high among high-income countries.

Leptospirosis is considered an occupational disease in New Zealand since it affects mainly people working in close contact with livestock species as farmers and abattoir workers. A previous investigation quantified sero-prevalence to *Leptospira borgpetersenii* serovar Hardjo (Hardjo-bovis) and *Leptospira interrogans* serovar Pomona (Pomona) and incidence of infection in abattoir workers. However, sero-prevalence in other occupational groups frequently exposed to livestock, and therefore at risk of leptospirosis (e.g. farmers and veterinarians), had not been assessed recently.

In New Zealand, most dairy cattle and pig herds are vaccinated annually against leptospirosis. Therefore, the risk of exposure and infection in people working in these production systems is thought to be limited. On the other hand, vaccination coverage in beef cattle, sheep and deer herds/flocks is still restricted, despite evidence of Hardjo-bovis and/or Pomona sero-positivity in most herds/flocks and

the potential for farmer's exposure to *Leptospira*. Therefore, we decided to target farmers of beef cattle, sheep and deer to quantify *Leptospira* sero-prevalence and assess risk factors for sero-positivity.

Animal vaccination is thought to be the most effective tool to reduce human exposure to *Leptospira*. Nevertheless, no formal assessment of vaccine efficacy to prevent urinary shedding in animals had been done previously. Therefore, we aimed to systematically review the literature and conduct a meta-analysis of vaccination trial results to quantify vaccine efficacy.

Globally, human disease is thought to be under-estimated due to non-specificity of signs and symptoms of disease, under-diagnosis and under-reporting. Efforts have been done internationally to estimate the number of leptospirosis cases worldwide. Nevertheless, no current estimate of the extent of leptospirosis under-ascertainment in humans and the effects of infection in both humans and animals was available in New Zealand.

In Chapter 1 of this thesis, a narrative literature review was performed covering animal and human sero-positivity and human notification reports in New Zealand, production effects attributed to *Leptospira* infection in beef cattle, sheep and deer of New Zealand, and *Leptospira* vaccination to reduce the risk of urinary shedding of leptospires in these species. In Chapter 2 and 3, *Leptospira* sero-positivity in farmers and veterinarians, factors associated with the serological status, and the associated infection risks were described and quantified. These studies, and results of earlier investigations of leptospirosis in abattoir workers and production limiting effects in deer, beef cattle and sheep, were jointly evaluated to estimate the burden and economic cost of leptospirosis in New Zealand (Chapter 4). In addition, the efficacy of animal vaccines was systematically reviewed and evaluated in a meta-analysis (Chapter 5). Main findings of this thesis, critiques to the methodology used, and areas of future research are discussed in Chapter 6.

Chapter 1

A review of leptospirosis in animals and people of New Zealand and livestock vaccination for control

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1.1 Summary

A decreasing trend in human leptospirosis notifications was observed from 2001 to 2014. Notwithstanding, human leptospirosis in New Zealand continues to be at one of the highest rates among high-income countries, affecting predominately people occupationally exposed to livestock species. Shedding of leptospires in the urine of livestock can be prevented by vaccination, and although in New Zealand there are eight vaccines registered for use in cattle, three for use in sheep and three for use in deer, vaccine coverage in beef cattle, sheep and deer is still limited.

Production losses potentially associated to *Leptospira* infection have been observed in New Zealand deer. Losses attributed to *Leptospira* infection have also been observed in some beef herds and sheep under specific conditions of high early-life challenge. It seems plausible that vaccination can prevent those losses but more research on the cost-benefit of vaccination is needed. Lastly, the potential benefits for the public health sector cannot be ignored.

Humoral immunity is important for preventing leptospirosis but also cell-mediated immunity appears to have a role in protecting cattle against Hardjo-bovis infection. Some monovalent Hardjo vaccines were observed to trigger a cell-mediated immune response. Nevertheless, no conclusive evidence exist to support that monovalent Hardjo vaccines confer a better level of protection against Hardjo-bovis challenge in cattle than multivalent vaccines containing serovar Hardjo.

The potential interference of maternally derived antibodies (MDA) with *Leptospira* vaccination has not been fully resolved. Nevertheless, under field conditions it is unlikely that MDA persist for more than four months in most animals. Recent evidence suggested that MDA appear to play a minor role for vaccine induced immune response interference. The answer to the question of when to vaccinate young stock may vary from farm to farm depending on specific conditions of colostrum management and onset of infection. Under an expected high level of challenge on a farm, vaccination of young animals before *Leptospira* exposure may be beneficial in preventing disease, reducing urinary shedding and human exposure.

In terms of vaccine efficacy to prevent shedding of leptospires in urine of cattle, sheep and deer; the evidence shows that vaccination of unexposed animals is

capable of preventing disease and subsequent urinary shedding from a moderate to high level of efficacy. An important consideration when assessing vaccine efficacy in preventing shedding is the method used to detect leptospires in urine, as PCR is more sensitive than culture or dark field microscopy. Individual animal responses to vaccination and potential individual vaccine failures should be overcome by the use of a herd vaccination programme.

1.2 Introduction

Leptospirosis is a neglected and re-emerging zoonotic disease of worldwide distribution. Typically a higher incidence is reported in tropical regions compared with temperate regions, often associated with poor socio-economic conditions (Levett 2001). Among developed countries and those with temperate climates, New Zealand has one of the highest rates of human notified cases of leptospirosis per year (Pappas et al. 2008), and it is often regarded as an occupational disease associated with livestock exposure since farmers and abattoir workers represent on average around 80% of notified cases each year (ESR 2002-2015).

Human leptospirosis in occupationally at risk groups is potentially preventable through livestock vaccination. Although vaccination coverage against *Leptospira* in the New Zealand dairy cattle population is already high at about 90% (Heuer 2009), in other livestock species as beef cattle, deer, and sheep; its use is still limited despite potential benefit for the farmer in reducing production losses as abortions, and mortality of young animals; and the public health benefit in reducing exposure to *Leptospira* and the number of people infected each year.

Earlier research into leptospirosis in New Zealand livestock was reviewed by Marshall and Manktelow (2002), and Ayanegui-Alcerreca et al. (2007) reviewed leptospirosis in New Zealand farmed deer. This paper reviews recent data on human leptospirosis notifications, *Leptospira* sero-positivity in humans and animals, production loss associated with leptospirosis in beef cattle, sheep and deer, and vaccines available for control in these species in New Zealand. The review also considered vaccination trials assessing vaccine efficacy to prevent shedding of leptospires in urine of cattle, sheep and deer, aspects of immunity after vaccination and infection, and the impact of MDA on vaccine efficacy to prevent shedding. A meta-analysis of vaccine efficacy to prevent shedding of leptospires in urine is presented in Chapter 5.

1.3 Materials and Methods

A narrative literature review on human and animal leptospirosis in New Zealand and production effects of leptospirosis in beef cattle, sheep and deer in the country was performed. Additionally, literature on induced immunity after *Leptospira* vaccination and infection, vaccine efficacy to prevent urinary shedding in cattle, sheep and deer, and potential interference of MDA with the effect of vaccination was also reviewed.

The online ACVM register of veterinary medicines, agricultural chemicals and vertebrate toxic agents (NZFSA 2015) was searched in September, 2015 for approved *Leptospira* vaccines of use in cattle, sheep and deer. Information on recommended use, serovars included, and interference with MDA was extracted from available labels and summarised.

Annual surveillance summaries of notifiable diseases in New Zealand from 2001 to 2014 (ESR 2002-2015) were reviewed to extract information on number of human notified cases, human leptospirosis annual incidence, serovars associated with infection, and occupation of leptospirosis cases. The trend of human leptospirosis notified cases from 2001 to 2014 was tested using the Mann-Kendall test. Incidence data were smoothed using local regression (LOESS) and span of 0.5, to visualise the trend in the last 14 years. Data analysis was performed using R version 3.3.0 (R Core Team 2015).

1.4 Human leptospirosis incidence and *Leptospira* sero-positivity in New Zealand

Before the introduction of vaccination in dairy cattle, leptospirosis was recognised as the number one occupational disease of dairy farming in New Zealand (Philip 1976). Prior to 1970, human leptospirosis was mostly associated with *Leptospira interrogans* serovar Pomona (Pomona) infection but the role of *Leptospira borgpetersenii* serovar Hardjo (Hardjo-bovis) was highlighted by Christmas et al. (1974) who isolated Hardjo-bovis for the first time in New Zealand from blood of acutely ill dairy farm workers. A serological survey in one region of New Zealand reported

that 34% of people milking cows had microscopic agglutination test (MAT) antibody titres ≥24 against Hardjo-bovis, Pomona, *Leptospira borgpetersenii* serovar Ballum (Ballum) and/or *Leptospira interrogans* serovar Copenhageni (Copenhageni) (Mackintosh et al. 1980a). An overall sero-prevalence of 44% MAT positive (MAT ≥24) to Hardjo-bovis, Pomona, *Leptospira borgpetersenii* serovar Tarassovi (Tarassovi), Ballum, Copenhageni and/or *Leptospira interrogans* serovar Australis (Australis) was later observed in a nationwide serological survey of 308 dairy farm workers (Blackmore and Schollum 1982). Comparatively lower sero-prevalences were observed at the time in other occupational groups at risk of leptospirosis such as meat inspectors (10%), abattoir workers (6%), veterinarians (8%) and farmers of beef cattle and sheep (8%) (Blackmore and Schollum 1983).

Human leptospirosis cases during the 1970s averaged 488 per year (11 per 100,000 people) but they decreased to 4.5 per 100,000 people in the early 1980s due to what was thought to be mainly the preventive effect of vaccination in dairy cattle (Marshall 1987). However, despite plausibility, lack of historical data on the timing of introduction and amount of vaccine used on farms hindered a causal inference on the association between the use of vaccination in dairy cattle and the reduction in human notified cases (Marshall and Chereshsky 1996). Attention to risk factors such as workplace smoking, and wearing personal protective equipment may have influenced the decline of human cases.

In recent years, the incidence of human leptospirosis in New Zealand has ranked high among developed countries with an average of 2.24 (95% CI 1.85–2.64) cases per 100,000 people a year during 2001-2014 (ESR 2002-2015). However, a significant (p=0.001) decreasing trend in the reported incidence of leptospirosis was observed during this period (Figure 1.1). Leptospirosis incidence in the last five years averaged 1.9 cases per 100,000 people a year, which still ranks high among high-income countries (Pappas et al. 2008; ECDC 2014).

Thornley et al. (2002) reported that the incidence of Hardjo-bovis and Pomona in leptospirosis notified cases in New Zealand from 1990 to 1998 decreased from 2.3 and 1.6 to 1.1 and 0.5 per 100,000 people, respectively; while the incidence of Ballum in these subjects increased in the same period from 0.2 to 0.6 per 100,000 people. Surveillance data from 2001 to 2014 showed that Hardjo-bovis was the most common serovar in human notified cases of New Zealand representing 46.3% of the

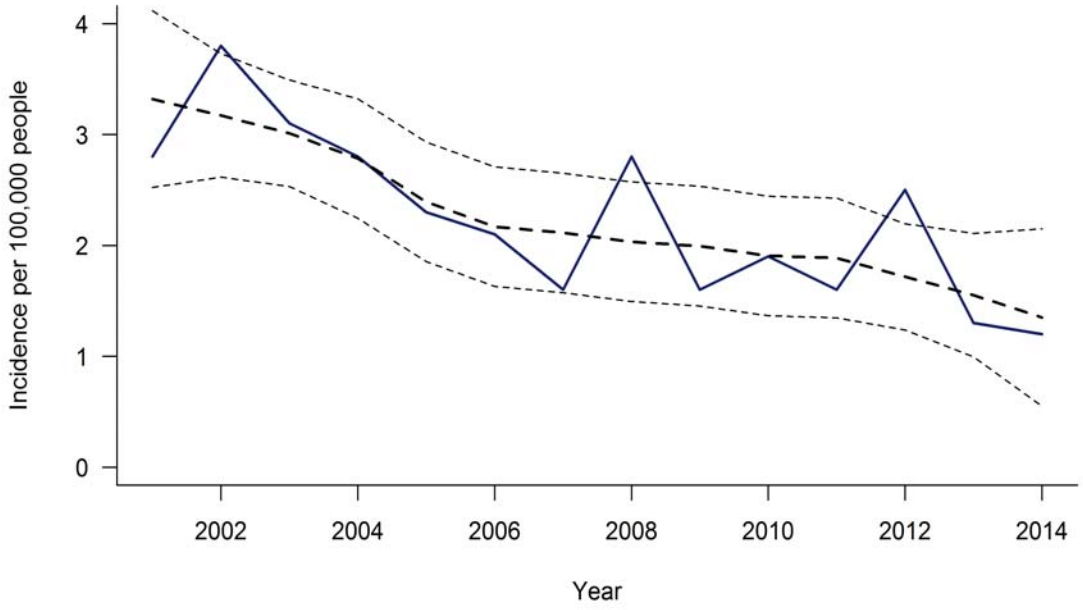

Figure 1.1: Number of New Zealand notified cases per 100,000 people a year. Dashed lines show the smoothed trend and 95% confidence intervals for the period 2001-2014 (ESR 2002-2015).

cases, followed by Pomona (23.3%), Ballum (17.5%), Tarassovi (7.5%), and Copenhageni (2.4%) of cases. An increase in the number of Ballum notified cases was observed since 2005, becoming the most frequent serovar in 2010-11 and the second on average after Hardjo-bovis since 2008 (ESR 2002-2015). Figure 1.2 shows the trend in the number of notified cases by serovar in the last 14 years.

Human leptospirosis in New Zealand continues largely as an occupational disease of people working in close contact with livestock species. During 2001 to 2014, the two most common occupations among notified cases were farmers and abattoir workers, representing on average 46.2% and 34.7% of the notified cases a year; respectively (Figure 1.3).

Recent serological surveys quantified the level of *Leptospira* sero-positivity in people working in at risk occupations. For example, a serological study in abattoir workers observed a sero-prevalence to Hardjo-bovis and Pomona of 10.9% (95% CI 8.5%–13.9%) using the MAT titre cut-off of $\geq$48 (Dreyfus et al. 2014); meanwhile a sero-prevalence (MAT $\geq$48) of 6.6% (95% CI 3.6%–10.9%) to Hardjo-bovis, Pomona,

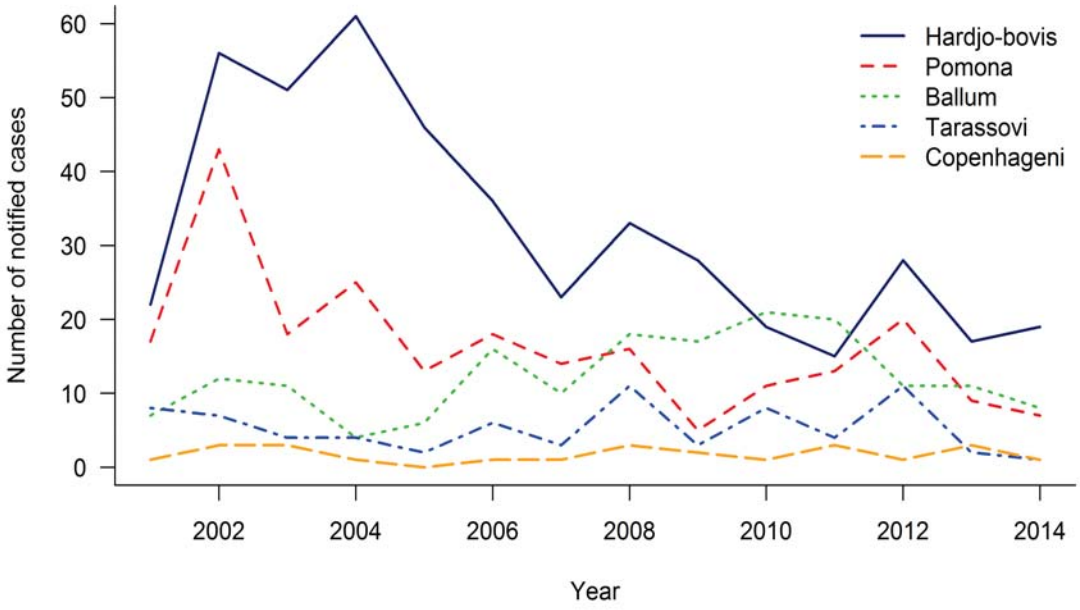

Figure 1.2: Number of notified cases by serovar in New Zealand from 2001 to 2014 (ESR 2002-2015).

Ballum, Tarassovi and Copenhageni was estimated in farmers of beef cattle, sheep and deer (Chapter 2). Similarly, a sero-prevalence of 5.1% (95% CI 2.8%–8.3%) to the same five serovars was estimated in veterinarians (Sanhueza et al. (2015), Chapter 3).

1.5 *Leptospira* sero-positivity in domestic animals and free-living species of New Zealand

Leptospira sero-positivity is widespread among beef cattle, sheep and farmed deer populations of New Zealand. Several serological surveys have been conducted over the years in these species. Overall, Hardjo-bovis and Pomona were the most frequently observed serovars at the individual animals and herd/flock level (Dreyfus et al. 2011; Subharat et al. 2012b). However, serological evidence of Ballum, and Copenhageni has also been reported in cattle, sheep and deer (Ris et al. 1973; Blackmore et al. 1982; Flint et al. 1988). In the latter species, serological evidence of *Leptospira borgpetersenii* serovar Arborea (Arborea) and Australis has also been reported (Wilson et al. 1998; Subharat et al. 2011a). Although past serological sur-

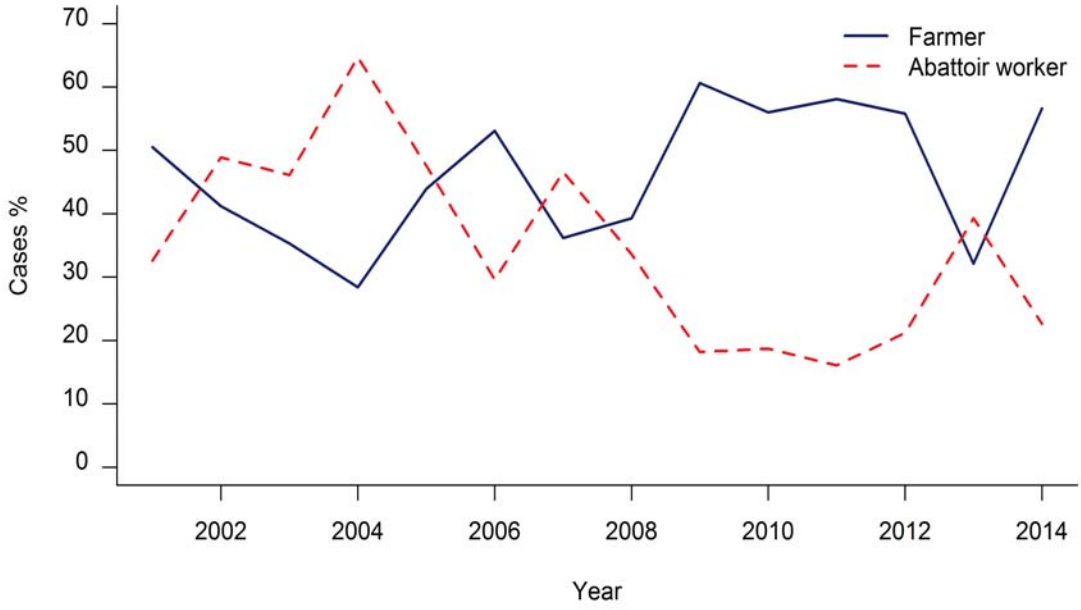

Figure 1.3: Percentage of notified cases working in the farming or abattoir industries from 2001 to 2014 (ESR 2002-2015).

veys have estimated animal and herd prevalence to different serovars in beef cattle, sheep and deer; often different MAT titre cut-offs were used to categorise an animal or a herd as positive and therefore sero-prevalence comparisons between studies need to take this into consideration. Moreover, the number of animals and herds used can be an indication of how robust the prevalence estimate was. Table 1.1 summarises sero-prevalence estimates for each of the serovars tested in beef cattle, sheep and deer at the animal and herd/flock level, the MAT titre cut-off used, and the number of animals and herds/flocks used by each study.

Serological surveys have also been conducted in dogs. Results consistently showed that Copenhageni was the most common serovar followed by Hardjo-bovis. The latter was associated with dogs from rural locations or breeds used as farm working dogs (O'Keefe et al. 2002; Harland et al. 2013), which suggests transmission from infected livestock to dogs working on the property. However, the role of free-living species cannot be completely ruled out; especially if we consider that in possums (*Trichosurus vulpecula*) *Leptospira borgpetersenii* serovar Balcanica, a serovar that cross-reacts in the MAT with Hardjo, is endemic (Hathaway et al. 1981; Cowan

et al. 1991; Horner et al. 1996). *Leptospira* exposure is also widely present in other wildlife species: serological evidence of Ballum was observed in 34% and 26% of free living *Rattus rattus* and *Rattus norvegicus*, respectively (Hathaway and Blackmore 1981), and in hedgehogs (*Erinaceus europaeus*), serological evidence of Ballum was also common (36%), followed by Copenhageni (16%) (Hathaway et al. 1981). Contrasting serological results in hedgehogs from dairy farms and Wellington city suggested that hedgehogs were an important reservoir for Ballum in dairy systems (Brockie and Till 1977). In feral goats, serological evidence (MAT $\geq$48) of Hardjobovis (11%) and Ballum (3%) was observed (Schollum and Blackmore 1981). In feral deer, serological evidence (MAT $\geq$50) of Pomona was observed in 10 of 24 deer tested (Inglis 1984), which contrasts with the results observed by Hathaway et al. (1981) who did not observe serological reactions in 27 wild deer tested; and Daniel (1966) who observed a sero-prevalence of 0.9% to Pomona (MAT $\geq$200) and 4.6% (MAT $\geq$10) in 109 wild deer tested. Geographical and environmental determinants for the distribution of wildlife infection are unknown. Further research on wildlife sero-positivity to *Leptospira* may contribute to elucidate the current role of these species on exposure of domestic livestock and humans.

Table 1.1: Sero-prevalence point estimates of beef cattle, sheep and deer at the animal and herd/flock level and cut-off used for a positive animal and herd.

		Animal-level			Herd level			
Serovar	**Species**	**MAT**	**n**	**(%)**	**cut-off**	**n**	**(%)**	**Author**
Hardjo-bovis	Sheep	1:48	396[a]	36	-	-	-	Fang et al. (2015)
Pomona	Sheep	1:48	396[a]	35	-	-	-	Fang et al. (2015)
Hardjo-bovis	Beef	1:48	146[a]	58	-	-	-	Fang et al. (2015)
Pomona	Beef	1:48	146[a]	25	-	-	-	Fang et al. (2015)
Hardjo-bovis	Deer	1:48	1107	25	4 positive	19	65	Subharat et al. (2012b)
Hardjo-bovis	Beef	1:48	767	44	4 positive	14	69	Subharat et al. (2012b)
Hardjo-bovis	Sheep	1:48	1244	31	4 positive	13	64	Subharat et al. (2012b)
Pomona	Deer	1:48	1107	13	4 positive	19	30	Subharat et al. (2012b)
Pomona	Beef	1:48	767	11	4 positive	14	21	Subharat et al. (2012b)
Pomona	Sheep	1:48	1244	3	4 positive	13	10	Subharat et al. (2012b)
Hardjo-bovis	Sheep	1:48	3361	43	1 positive	162	91	Dreyfus et al. (2011)
Pomona	Sheep	1:48	3361	14	1 positive	162	74	Dreyfus et al. (2011)
Hardjo-bovis	Beef	1:48	2308	50	1 positive	116	92	Dreyfus et al. (2011)
Pomona	Beef	1:48	2308	25	1 positive	116	72	Dreyfus et al. (2011)
Hardjo-bovis	Deer	1:48	1992	26	1 positive	99	60	Dreyfus et al. (2011)
Pomona	Deer	1:48	1992	11	1 positive	99	49	Dreyfus et al. (2011)
Hardjo-bovis	Deer	1:24	2016	61	3 positive	111	78	Ayanegui-Alcerreca et al. (2010)
Pomona	Deer	1:48	2016	8	3 positive	111	20	Ayanegui-Alcerreca et al. (2010)

Copenhageni	Deer	1:48	2016	1	3 positive	111	0	Ayanegui-Alcerreca et al. (2010)
Hardjo-bovis	Sheep	1:48	2758[a]	5	-	-	-	Dorjee et al. (2008)
Pomona	Sheep	1:48	2758[a]	1	-	-	-	Dorjee et al. (2008)
Hardjo-bovis	Deer	1:50	417	18	-	-	-	Reichel et al. (1999)
Pomona	Deer	1:50	417	2	-	-	-	Reichel et al. (1999)
Copenhageni	Deer	1:50	417	0.2	-	-	-	Reichel et al. (1999)
Hardjo-bovis	Deer	1:96	-	-	1 positive	53	74	Wilson et al. (1998)
Pomona	Deer	1:96	-	-	1 positive	53	42	Wilson et al. (1998)
Copenhageni	Deer	1:96	-	-	1 positive	53	11	Wilson et al. (1998)
Tarassovi	Deer	1:96	-	-	1 positive	53	15	Wilson et al. (1998)
Ballum	Deer	1:96	-	-	1 positive	6	17	Wilson et al. (1998)
Australis	Deer	1:96	-	-	1 positive	6	33	Wilson et al. (1998)
Balcanica	Deer	1:96	-	-	1 positive	6	66	Wilson et al. (1998)
Hardjo-bovis	Deer	1:10	360	39	-	-	-	Flint et al. (1988)
Pomona	Deer	1:10	360	14	-	-	-	Flint et al. (1988)
Ballum	Deer	1:10	360	47	-	-	-	Flint et al. (1988)
Tarassovi	Deer	1:10	360	26	-	-	-	Flint et al. (1988)
Copenhageni	Deer	1:10	360	43	-	-	-	Flint et al. (1988)
Australis	Deer	1:10	360	13	-	-	-	Flint et al. (1988)
Bratislava	Deer	1:10	360	53	-	-	-	Flint et al. (1988)
Hardjo-bovis	Deer	1:32	27	22	-	-	-	Flint (1985)
Pomona	Deer	1:32	27	0	-	-	-	Flint (1985)

Ballum	Deer	1:32	27	0	-	-	-	Flint (1985)
Copenhageni	Deer	1:32	27	0	-	-	-	Flint (1985)
Tarassovi	Deer	1:32	27	0	-	-	-	Flint (1985)
Hardjo-bovis	Sheep	1:48	928	20	No stated	43	64	Blackmore et al. (1982)
Pomona	Sheep	1:48	928	4	No stated	43	51	Blackmore et al. (1982)
Ballum	Sheep	1:48	928	3	No stated	43	42	Blackmore et al. (1982)
Tarassovi	Sheep	1:48	928	3	No stated	43	29	Blackmore et al. (1982)
Copenhageni	Sheep	1:48	928	2	No stated	43	22	Blackmore et al. (1982)

[a] Slaughterhouse samples.

1.6 Production effects associated with *Leptospira* infection in New Zealand livestock

A more extensive review on production effects worldwide due to leptospirosis in livestock was done by (Vallée et al. 2016a). To date, there is no evidence of *Leptospira interrogans* serovar Hardjo (Hardjo-prajitno) presence in New Zealand. This section will focus mainly on production losses attributed to Hardjo-bovis and Pomona infection in New Zealand beef cattle, sheep and deer.

1.6.1 Cattle

Pomona has long been recognised as a cause of sporadic abortion in cattle (Te Punga and Bishop 1953) but the effect of the host adapted strain Hardjo-bovis on pregnant cattle has been controversial. Abortion investigations carried out in the 1970s in dairy cattle in the Waikato region successfully isolated Pomona, and not Hardjo-bovis, from cows that had aborted, while solely Hardjo-bovis, and not Pomona, was isolated from herds not experiencing abortions, which suggested that Hardjo-bovis was not a cause of abortion in these herds (Carter et al. 1982). Recently though, both Hardjo-bovis and Pomona were associated serologically with abortions in beef cattle comparing aborting with non-aborting cows from the same farm (Sanhueza et al. 2013). Serological assessment of the role of *Leptospira* on abortions in cattle is difficult mainly due to the variability of MAT antibody responses in naturally exposed animals. Outside New Zealand for example, Ellis et al. (1982b) found that 22.8% of cows aborting *Leptospira* infected foetuses, had no detectable (<1:10 MAT) antibody titres. In another study, Ellis et al. (1986) artificially challenged 22 pregnant heifers with Hardjo, resulting in 8/22 apparently healthy calves and 14/22 that had a range of negative outcomes: dead calves (n=6), stillborn (n=1), mummified (n=1), extremely weak calves that died soon after birth (n=3), and weak calves that survived (n=3). These stood in contrast to seven unexposed heifers, which all produced healthy calves. Leptospires have also been isolated from kidneys of apparently healthy sero-negative bovine foetuses, which lead to the hypothesis that calves were infected before developing immune-competence, or else they developed immune-tolerance to *Leptospira* antigens. This is important since it highlights that infection during pregnancy could result in congenitally and perhaps permanently infected calves that would shed *Leptospira* in the urine into the envi-

ronment, potentially exposing other farm animals, humans and/or wildlife species (Ellis et al. 1982a; Giles et al. 1983; Bolin et al. 1989b). As an example, Ellis et al. (1986) reported one calf with a MAT titre of 1:30,000 to Hardjo before the first colostrum intake from which leptospires were recovered from kidneys when slaughtered 59 days after birth. However, there is little evidence about the frequency of such events in the population.

In a vaccination trial conducted on seven New Zealand beef farms, Vallée et al. (2014) observed a borderline significant (p=0.07) difference in live weight of 8kg and 14 kg between vaccinated and unvaccinated 14 - 19 months heifers, on two farms with high natural Hardjo-bovis challenge (sero-prevalence=76%). No difference in calving or weaning rate between vaccinated and unvaccinated heifers was observed by the same authors.

1.6.2 Sheep

Reports of mortality events presumably caused by leptospirosis were first reported during the 1950s (Hartley 1952). Outbreak investigations of mortality in lambs on three sheep farms also attributed losses to Pomona infection (Vermunt et al. 1994). Hodges (1974) challenged six lambs intravenously with Pomona and observed a wide range of clinical outcomes: while four lambs developed haemoglobinuria and haematuria, of which two died five - six days after challenge, the other two did not showed signs of infection.

Pomona infection can cause abortions in ewes as reported on a New Zealand sheep farm where a strong and significant association (OR=13.77, p<0.001) between foetal loss and sero-positivity (MAT $\geq$48) for Pomona was observed (Ridler et al. 2015). On this farm, the odds of foetal loss were higher in non-vaccinated compared with vaccinated ewes (OR=1.89; p=0.01). Vallée et al. (2016b) observed a significant difference in weaning rates of up to 22.6 percentage points between Hardjo-bovis sero-positive hoggets (MAT $\geq$1536) and Hardjo-bovis and Pomona sero-negative hoggets (MAT <1536). Overall, Hardjo-bovis sero-positive hoggets had lower odds of having a lamb at weaning than Hardjo-bovis or Pomona sero-negative hoggets (OR=0.41, 95% CI 0.19–0.93).

Webster and Reynolds (1955) observed that unvaccinated lambs challenged intraperitoneally with Pomona had on average a lower weight compared to vaccinated lambs but significance was not assessed. A vaccination trial on seven farms showed no overall difference in growth rate between vaccinated and unvaccinated animals. However, on one farm where natural Hardjo-bovis challenge occurred early in the season, there was a difference of 12g/day in favour of vaccinated sheep up to 14 weeks of age (Vallée et al. 2013).

1.6.3 Deer

Howell (1991) reported a mortality outbreak of 55 animals on one farm that was attributed to Pomona. It was observed that mortality was higher among young deer. Leptospires were demonstrated in the urine of a yearling stag and kidneys of a two-year-old animal. Both animals showed signs of chronic nephritis. In another mortality outbreak investigation on a deer farm, severe chronic nephritis was also observed in deer calves that died presumably due to Pomona infection (Fairley et al. 1984; Fairley et al. 1986).

Subharat et al. (2012a) conducted a vaccination trial on four deer farms under natural Hardjo-bovis challenge. They found that a reduced average daily gain (bodyweight) of 31g/d in unvaccinated controls compared with vaccinated animals over 96 days, suggesting that Hardjo-bovis infection reduced growth rate of unvaccinated deer. Similarly, Ayanegui-Alcerreca (2006) observed that deer without serological evidence of Hardjo-bovis or Pomona were on average 3.7kg heavier than exposed animals but no difference was observed between vaccinated and control deer, possibly because many vaccinated deer were infected prior to vaccination. Subharat et al. (2011b) reported that vaccinated primiparous hinds had a mean weaning rate 7% (6% absolute difference) higher than unvaccinated hinds in four farms with serological evidence of Hardjo-bovis challenge. Similarly, Ayanegui-Alcerreca (2006) observed a 10% higher weaning percentage (9% absolute difference) in vaccinated hinds compared to unvaccinated hinds under dual Hardjo-bovis and Pomona challenge.

1.7 Vaccines registered for use in cattle, deer and sheep in New Zealand

Individual vaccine recommendations, species registered for, and serovars included in commercial products available for use in New Zealand are summarised in Table 1.2. In 2015, there were eight leptospiral vaccines registered for use in at least one of these species (cattle, sheep, or deer) to prevent urinary shedding or as an aid in the control of leptospirosis (Table 1.2). Four vaccines are registered for use in cattle only; two are registered for use in cattle, sheep and deer; one for cattle and deer; and one for cattle and sheep (NZFSA 2015). All vaccines include serovars Hardjo and Pomona, and three vaccines include also serovar Icterohaemorrhagiae or Copenhageni; two cross protecting serovars. Two of these vaccines that contain Icterohaemorrhagiae or Copenhageni are approved for use in cattle only and one for use in cattle and deer.

Vaccine labels advise that a first vaccination must be followed by a second dose administered after four to six weeks. To maintain immunity, a single annual booster is required. It is also recommended on most labels to vaccinate before the period of high risk of leptospirosis that generally goes from autumn to early summer, and to administer the booster vaccination prior to parturition in order to increase antibody levels in colostrum, potentially improving the protection of new-born calves against *Leptospira* challenge. Most products warn that maternal antibodies may interfere with vaccination response (see Maternally derived antibodies (MDA) and vaccination section). Although vaccination of young animals is not explicitly labelled for all products, a booster at about six months of age is recommended if calves were to be vaccinated at a young age.

1.8 Vaccine coverage in cattle, sheep and deer in New Zealand

In contrast to dairy cattle where it is estimated that 90% of herds are annually vaccinated against leptospirosis (Heuer 2009), vaccination of beef cattle, sheep and deer is not routinely performed in most herds or flocks. It was observed that between 18%-42% of beef, 5%-13% of deer and 0.6%-1% of sheep farms vaccinate against leptospirosis (Wilson et al. (2008), Heuer (2009), Dreyfus et al. (2011), and Chapter 2).

As described in a previous section, an increase in vaccination of dairy cattle during the early 1980s was followed by a reduction of human cases (Marshall 1987). Although increased awareness may have also played a role in human leptospirosis prevention, it is plausible that an increase of vaccine coverage in beef cattle, sheep and deer could potentially reduce the incidence of leptospirosis in people who are in regular contact with these species. The main factor limiting the widespread use of *Leptospira* vaccines in these species is the cost-benefit of vaccination, which may be particularly low for sheep (Keenan 2007). Consequently only a small proportion of sheep flocks are currently vaccinated against leptospirosis. Recent findings have suggested that vaccination could improve weight gain in some beef cattle and sheep farms under certain circumstances (i.e. infection at a young age) (Vallée et al. 2014). In deer on the other hand, production losses potentially attributed to leptospirosis have been consistently observed (Ayanegui-Alcerreca 2006; Subharat et al. 2011b; Subharat et al. 2012a). Vaccination against leptospirosis in deer may be a profitable option for the farmer depending on the risk of infection that animals are subjected to (Wilson et al. 2009). On top of that, the potential benefit on public health should be considered but more research is required to quantify this effect and the cost-benefit of vaccination.

Table 1.2: Vaccines registered in New Zealand to prevent leptospirosis in cattle, sheep and deer animals as at October 2015 (NZFSA 2015).

Trade name	**Species**	**Serovars**	**Administration**	**Registrant**
Leptavoid 2	Cattle, Sheep, Deer, Pigs	Hardjo, Pomona	**Use**: For active immunisation against *Leptospira*. Vaccination of healthy cattle will prevent urinary shedding for 12 months. Vaccination will not alter the shedding status of infected animals. **Primary**: Two subcutaneous doses 4 to 6 weeks apart. **Calves**: Maternal antibodies may interfere with the response to vaccination if administered before 6 months of age. If primary vaccination is completed before 6 months of age, a booster is required once they reach 6 months of age. **Booster**: Subcutaneous dose within 12 month after primary vaccination and annually thereafter, ideally prior to parturition to maximise maternal antibodies.	MSD

Leptavoid 3	Cattle, Deer	Hardjo, Pomona, Icterohaemorrhagiae[a]	**Use**: For active immunisation against *Leptospira*. Vaccination of healthy cattle will prevent urinary shedding for 12 months. **Primary**: Two subcutaneous doses 4 to 6 weeks apart. **Calves**: Maternal antibodies may interfere with the response to vaccination if administered before 6 months of age. If primary vaccination is completed before 6 months of age, a booster is required once they reach 6 months of age. **Booster**: Subcutaneous dose within 12 month after primary vaccination and annually thereafter, ideally prior to parturition to maximise maternal antibodies.	MSD
Cattlevax	Cattle	Hardjo, Pomona, Clostridiums	**Use**: For active immunisation against leptospirosis. Vaccination of healthy cattle before infection will prevent urinary shedding of *Leptospira*. Vaccination will not alter the shedding status of infected animals. **Primary**: Two subcutaneous doses 4 to 6 weeks apart. Vaccination should be completed 2 weeks prior to the period of risk. **Calves**: Maternal antibodies may interfere with the response to vaccination if administered before 6 month of age. Calves in high risk areas may be vaccinated form 4 weeks of age. A booster is essential at 6 months of age. **Booster**: Annual single dose or every 6 months in areas where clostridial challenge is high.	MSD

Lepto 2-Way	Cattle	Hardjo, Pomona	**Use**: To prevent Hardjo and Pomona shedding in urine of cattle. Primary: Two subcutaneous doses 4 to 6 weeks apart. **Calves**: Early vaccination is advisable. Vaccination may begin from 12 weeks of age. It is essential a booster at 6 to 9 months of age. **Booster**: Single annual dose each autumn.	Virbac
Lepto 3-Way	Cattle	Hardjo, Pomona, Icterohaemorrhagiae[a]	**Use**: To prevent shedding in urine of serovars contained in vaccine. **Primary**: Two subcutaneous doses 4 to 6 weeks apart. **Calves**: Early vaccination is advisable. Vaccination may begin from 12 weeks of age. It is essential a booster at 6 to 9 months of age. **Booster**: Single dose annually each autumn.	Virbac

Leptoshield	Cattle, Sheep, Goats, Deer	Hardjo, Pomona	**Use**: For the prevention of leptospirosis in cattle, sheep and goats. As an aid in the control of leptospirosis in deer. For the prevention of urinary shedding of Hardjo and Pomona in healthy cattle and protection against reproductive losses in cattle. **Primary**: Two subcutaneous doses 4 to 6 weeks apart, before season of high risk (autumn to early summer). **Calves**: Early vaccination of calves is advisable. Effective in presence of maternal antibodies. Calves may be vaccinated from 1 month of age. If primary vaccination is completed before 3 months of age, a booster is required 6 months later. **Deer calves** should commence a vaccination program at 3 months of age. **Booster**: Annually, breeding females about 1 month before calving.	Zoetis

Ultravac 7in1	Cattle, Sheep	Hardjo, Pomona, Clostridiums	**Use**: For the prevention of leptospirosis in cattle. Prevents urinary shedding of *Leptospira* when administered prior to exposure. Protection of cattle against reproductive losses. **Primary**: Two subcutaneous doses 4 to 6 weeks apart. Vaccinate before period of high risk (autumn to early summer). **Calves**: Early vaccination of calves is advisable. Effective in presence of maternal antibodies. Calves may be vaccinated from 1 month of age. If primary vaccination is completed before 3 months of age, a booster is required 6 months later. **Lambs**: Efficacious in lambs from 10 weeks of age. **Booster**: Annually preferably about 1 month before calving or at drying off to pass on immunity to the offspring.	Zoetis
Leptoshield 3	Cattle	Hardjo, Pomona, Copenhageni	**Use**: For protection against *Leptospira* infection in cattle. **Primary**: Two subcutaneous doses 4 to 6 weeks apart. Before period of high risk (usually autumn to early summer). **Calves**: Early vaccination of calves is advisable. Effective in presence of maternal antibodies. Calves may be vaccinated from 1 month of age. If primary vaccination is completed before 3 months of age, a booster is required 6 months later. **Booster**: Annually, before period of high risk (autumn to early summer).	Zoetis

[a] Provides immunity against serovar Copenhageni

1.9 Induced immunity to *Leptospira* vaccination and infection

The activation of the immune system involves different mechanisms in response to a vaccine or pathogen. These mechanisms usually function simultaneously but they can be conveniently divided into humoral and cell-mediated responses, according to the type of cells involved.

1.9.1 Humoral immune response

Humoral immune response or antibody-mediated immunity refers to the production of immunoglobulins against specific antigens. During *Leptospira* infection, the first immunoglobulins to rise are of class M (IgM). These are detectable from three to eight days, with peak titres at about 14 days after initial infection. This is followed by an increase in IgG titres from three to four weeks after infection (Leonard et al. 1992; Faine et al. 1999).

The MAT is widely used to detect antibodies against *Leptospira*. Its major advantage is that it provides evidence of the infecting serovar but cross reactions between closely related serovars, such as Hardjo and Balcanica (Hathaway et al. 1981), can hinder accurate diagnosis. MAT cannot differentiate between different classes of antibodies (Faine et al. 1999) or between vaccinated and infected animals (Ris and Hamel 1982; Ellis et al. 2000). During the first stages of infection, the interpretation of MAT results can be challenging due to cross reactions between leptospires of different serogroups; the specificity of the test is relatively higher in convalescent samples compared to acute samples (Levett 2001). Cross-protection between *Leptospira interrogans* serovar Hardjo type Hardjo-prajitno vaccination and Hardjo-bovis challenge, two different species that are antigenically related, has been observed in cattle (Ellis et al. 2000).

Some studies have observed that antibody titres induced by vaccination were in general lower in magnitude than titres induced by infection. For example, Allen et al. (1982) observed Hardjo-bovis MAT titres ranging from 32 to 512 in vaccinated animals compared with a range of 128 to 8192 in naturally infected unvaccinated animals. Similarly, Mackintosh et al. (1980b) observed a slightly lower geometric

mean MAT titre for vaccinated (135) compared with unvaccinated (175) cattle after natural challenge.

Agglutinating antibody titres determined by the MAT and induced by vaccination in cattle can last for variable amounts of time according to the vaccine and serovars used (Arduino et al. 2009). For Hardjo, mean MAT titres were observed to decline below 1:100 at 20 weeks post vaccination in cattle (Ellis et al. 2000), while others have observed undetectable titres (<1:24) after 17 weeks of vaccination (Mackintosh et al. 1980b) or titres remaining at 1:100 for about one year from vaccination (Zuerner et al. 2011). In cattle naturally infected with Hardjo-bovis, antibody titres were observed to last for at least 48 weeks after infection (Mackintosh et al. 1980b) but continued re-exposure cannot be ruled out under uncontrolled settings. Marshall et al. (1979a) observed Hardjo-bovis titres of 96 for at least 15 weeks after infection. Recently in sheep, the half-life of antibodies after natural infection was estimated as 6.7 months for Hardjo-bovis and 6.3 months for Pomona (Vallée et al. 2015). The persistence of measurable titres may depend on the ultimate titre induced by infection or vaccination.

A moderate or absent anamnestic MAT response was usually observed in vaccinated cattle after Hardjo challenge, contrasting with a comparatively stronger antibody response observed in unvaccinated cattle when challenged with Hardjo-bovis (Bolin and Alt 2001; Zuerner et al. 2011; Rinehart et al. 2012). This could be the result of a rapid elimination of leptospires from the animal, reducing the amount of antigen available to trigger antibody production. Nevertheless, lack of anamnestic response in vaccinated cattle after challenge was not always related to the shedding prevention of leptospires in urine (Bolin et al. 1989a), indicating that an effective immune response, at least for Hardjo-bovis, depends also on cellular immunity (Ellis et al. 2000).

Although antibody titres in animals demonstrate previous *Leptospira* infection or vaccination, they do not always correlate with resistance to challenge, and hence duration of immunity. A number of studies have observed that vaccines were capable of preventing shedding of leptospires in the absence of detectable MAT antibodies (Hoag and Bell 1955; Ris and Hamel 1979), but others have demonstrated lack of protection against Hardjo challenge and urinary shedding in the presence of MAT antibodies (Bolin et al. 1989a; Bolin et al. 1991; Zuerner et al. 2011).

Monovalent vaccines that stimulate both humoral and cell-mediated response have been shown to protect cattle against infection and shedding of leptospires in urine in contrast to vaccines that only stimulate a humoral response (Bolin et al. 1989a; Bolin and Alt 2001).

1.9.2 Cell-mediated immunity

Cell-mediated immunity refers to the direct action of cells, rather than antibodies, in the immune response. Whereas humoral immunity is important in limiting *Leptospira* infection in most species, cell-mediated immunity appears to have an important role in cattle exposed to serovar Hardjo-bovis (Adler and De la Peña Moctezuma 2010).

Naiman et al. (2001) observed a recall response characterised by *in vitro* proliferation of peripheral blood mononuclear cells (PBMC) after contact with Hardjo-bovis in cattle previously vaccinated with a monovalent Hardjo vaccine containing aluminium hydroxide as adjuvant. It was also observed that PBMC obtained from previously vaccinated cows exhibited a significantly higher production of interferon-γ when cultured with Hardjo-bovis antigen, compared to PBMC obtained from unvaccinated cows. Interferon-γ producing cells involved in this recall response were mainly identified as CD4+ T and $\delta\gamma$ T lymphocytes (Naiman et al. 2001; Naiman et al. 2002). Blumerman et al. (2007) observed that proliferation of $\delta\gamma$ T cells depended on direct or indirect contact with CD4+ T cells, and that the *in vitro* recall response of these cells was maintained from a minimum of one year to more than two years in some cows. Brown et al. (2003) observed a similar recall response of CD4+ T, CD8+ T, and $\delta\gamma$ T cell proliferation when comparing two monovalent vaccines containing aluminium based adjuvants in cattle but not in animals vaccinated with a pentavalent *Leptospira* vaccine containing a different adjuvant plus serovars Hardjo, Pomona, Canicola, Icterohaemorrhagiae and Grippotyphosa. This suggests an important role of vaccine adjuvant on protection against Hardjo-bovis. Zuerner et al. (2011) showed that not only CD4+, CD8+, and $\delta\gamma$ T cells were involved in response to Hardjo-bovis challenge but they also observed a recall response of natural killer cells (CD335+) characterised by interferon-γ production in cattle previously immunised with a Hardjo-bovis monovalent vaccine. However, prevention of Hardjo-bovis

shedding was not achieved completely.

In vitro proliferation of PBMC and production of interferon-γ was also observed in sera of cattle previously vaccinated with a Hardjo monovalent vaccine when cultured with serovar Grippotyphosa (Brown et al. 2003). Hence, cross protection is potentially plausible but it was not tested in this trial.

1.10 Maternally derived antibodies (MDA) and vaccination

At birth bovines are immunologically naïve, therefore unable to defend themselves against infections without the protection that colostrum may confer (Barrington and Parish 2001). Under natural challenge conditions, Cacchione et al. (1969) observed that 2.3% (2/88) of calves born from sero-positive dams had detectable antibodies ($\geq$1:100 MAT titre) to *Leptospira* before colostrum consumption. Colostrum contains immunoglobulins, mainly IgG1 (Stelwagen et al. 2009), that are absorbed by the permeable gut of the new-born to help to prevent infections during the first days of life. The absorption of MDA is mainly limited to the first 48 hours of life (Chase et al. 2008) and the concentration of IgG in colostrum decreases quickly during the first 14 hours after calving (Moore et al. 2005). Nevertheless, if vaccination is performed at a young age, there is the possibility that MDA interfere with the protective effect of vaccination (Chase et al. 2008). Questions then are i) when the first exposure is likely to occur (seasonal effects) and ii) how early calves and lambs can be vaccinated for the first time against leptospirosis.

The duration of the colostral antibodies in the neonate depends on the size of the initial titre and how long antibodies survive in the body before being catabolised. The half-life of IgG1 was estimated by Nielsen et al. (1978) to be around 18 days. McDonald and Rudge (1957) observed *Leptospira* specific MDA to last for more than two months in unchallenged calves. Palit et al. (1991) showed that MDA in calves born from dams vaccinated during pregnancy could be detected for up to three months after birth. Hellstrom (1978) found that 75% of calves had detectable MDA antibodies (MAT $\geq$17) against Hardjo-bovis and Pomona 50 days after birth. The percentage was reduced to 50% by 100 to 110 days and to 25% at 130 to 140

days of age. After 190 days no calf had detectable antibodies against *Leptospira*. It was also found that Pomona MDA were lower in magnitude and were detectable for shorter periods than Hardjo-bovis titres. No calf was sero-positive (MAT $\geq$24) against Pomona after 80 days. Under especial conditions however, Cacchione et al. (1969) found that MDA against *Leptospira* (MAT $\geq$100) could be maintained for 56 days in 81% of calves fed with a mixture of milk from sero-positive and sero-negative dams.

Some vaccine manufacturers recommend vaccinating pregnant cows close to calving in order to maximise antibodies in colostrum and protect new-born calves during the early life through colostrum consumption (Table 1.2). One of the first experiments to test the effects of dam vaccination on resistance of calves against *Leptospira* challenge was carried out by McDonald and Rudge (1957). They observed that calves receiving colostrum from vaccinated dams were protected against Pomona challenge at less than one month of age compared with calves born from unvaccinated sero-negative mothers.

It is generally recommended that calves should be vaccinated in the time window after maternal antibodies have fallen and before the occurrence of natural challenge, since vaccination has little or no effect on reducing shedding of already infected animals (Hancock et al. 1984). The onset of infection can vary from farm to farm, or from year to year. Circumstantial evidence suggested that management factors and weather may contribute to the spread of leptospirosis in a herd/flock. For example, it was reported that a leptospirosis outbreak in deer was preceded by the purchase of 70 bulls from 10 farms three months earlier. Deer on the farm were rotationally grazed two-six days behind the bulls (Fairley et al. 1984). Other outbreaks of leptospirosis in deer were observed after periods of close contact between animals such as yarding, transportation, and densely reared populations on a paddock (Howell 1991). An outbreak of leptospirosis in lambs in the Gisborne region occurred after shearing ewes and lambs, and a period of wet weather (Hartley 1952). In a cohort of sheep in eight farms of New Zealand, Vallée et al. (2015) observed that most sero-conversions to serovar Hardjo-bovis occurred during winter when sheep were between 10 to 15 months old.

Although the duration of MDA against *Leptospira* in groups of animals on a farm may depend on the management of colostrum supply, the timing of prior chal-

lenge or vaccination of the dam, and the infecting serovar, no conclusive evidence exists to date to support whether MDA against *Leptospira* interfere with the effect of vaccination. Gillespie and Kenzy (1958a) suggested that the level of immunity in calves induced by vaccination depended mainly on the age at first vaccination, since sero-negative calves vaccinated at one-two months of age and artificially challenged with Pomona, showed poorer protection against urinary shedding than sero-negative heifers vaccinated at six-eight months old. In the same context, Schollum and Marshall (1985) observed a higher serological response to vaccination in six-month-old calves compared to three-month-old calves, but urinary shedding after challenge was not assessed. On the other hand, Palit et al. (1991) suggested that the magnitude of MDA at vaccination did not influence vaccine efficacy to prevent urinary shedding (assessed by dark field microscopy and culture) in calves after Hardjo-bovis artificial challenge. Nevertheless, the low number of animals used prevented statistical significance of results. In a recent study conducted by Zimmerman et al. (2013), sero-negative calves (MAT<100) that had received colostrum from vaccinated mothers were vaccinated against Hardjo at four weeks of age and then artificially challenged a year later with Hardjo-bovis by conjunctival inoculation. Urinary shedding of leptospires was detected in 4/18 vaccinated and 18/18 unvaccinated calves (78% vaccine efficacy). Nevertheless, it is unknown whether the calves had any detectable MDA below the MAT cut-off used.

1.11 Vaccine efficacy to prevent shedding of leptospires in urine

Vaccine efficacy trials commonly differ in the species used, type of vaccine, age of animals, type of challenge, route of challenge, time from vaccination to challenge, and test used for detection of leptospires in urine. Hence, findings and inferences from those trials are difficult to compare. Moreover, publication bias towards successful protection and consequently high vaccine efficacy cannot be ruled out. Nevertheless, there is a substantial body of evidence supporting that leptospiral vaccines are efficacious for the prevention of infection and shedding of leptospires in urine. The level of shedding prevention by vaccination varied in each study. A meta-analysis of vaccine efficacy to prevent shedding of leptospires in urine of cattle, sheep and deer using data from this review estimated vaccine efficacy as 82.1% (95% CI 71.2–88.9)

when shedding was assessed by culture (Chapter 5).

A critical point in the evaluation of vaccine efficacy is the method used for detecting leptospires in urine. Culture has been extensively used in the past but its sensitivity is deemed to be low compared to polymerase chain reaction (PCR) or fluorescent antibody (FA), especially in vaccinated animals where leptospires may be shed in low numbers, which reduces the probability of isolation (Smith et al. 1994). Therefore, the use of culture as the only method to assess vaccine efficacy may bias results towards high vaccine efficacy (Alt et al. 2012). Zuerner et al. (2011) observed that vaccine efficacy depended on the method used for shedding assessment (culture, FA, or PCR). The PCR in this case detected *Leptospira* DNA in urine of vaccinated animals when culture or FA was negative, and consequently resulted in a lower apparent vaccine efficacy. PCR assays detect bacterial DNA, which may be from dead or live leptospires. Hence the interpretation of PCR for evaluating *Leptospira* vaccines is controversial. However, the observation of leptospiral DNA in urine for several weeks suggests active infection and successful renal colonisation.

A summary of vaccination trial results conducted in cattle, sheep and deer is now presented for each species:

1.11.1 Cattle

Early studies in cattle showed the potential of vaccination to prevent urinary shedding after artificial challenge with Pomona. Hoag and Bell (1955) conducted two vaccination trials in six-twelve month old calves. They vaccinated either once or twice 17 sero-negative calves against Pomona. Between one and two months after vaccinating calves, both vaccinated and unvaccinated animals were challenged intramuscularly with Pomona. The combined results showed that no leptospires were isolated from urine of vaccinated calves in contrast to unvaccinated animals, all of which had leptospires isolated from urine. Similar results were also observed in other vaccination trials under controlled settings and artificial Pomona challenge (Stalheim 1968; Ris and Hamel 1979). Marshall et al. (1982) vaccinated twice, one month apart, 11 calves of six months of age with a commercial Hardjo-Pomona vaccine. Nineteen days after the second vaccine dose, vaccinated and unvaccinated calves were challenged subcutaneously with Pomona. Leptospires were not isolated

from urine of vaccinated animals in contrast with 8/10 unvaccinated animals that had leptospires recovered from urine.

Vaccination did not always confer complete protection against Pomona infection after artificial challenge. Gillespie and Kenzy (1958a) observed that vaccinated cattle, when shedding leptospires urine, shed fewer and had less severe clinical signs compared with unvaccinated controls. This was also observed by some subsequent studies using artificial Pomona challenge (Gillespie and Kenzy 1958b; Ris 1977).

The focus of the research on *Leptospira* vaccination changed during the 1970s from Pomona challenge trials to Hardjo challenge trials. Tripathy et al. (1976) assessed the efficacy of three bacterins (bivalent, trivalent and pentavalent) to protect cattle against artificial Hardjo challenge. For the bivalent and trivalent formulation, two doses of the vaccine were given six months apart. For the pentavalent, two doses were given one month apart. Vaccinated and control animals were exposed to Hardjo intraperitoneally at 1.5 years old (11 months after first dose for bivalent and trivalent, and 7 months after first dose for pentavalent). *Leptospira* was not isolated from the kidneys of the 15 vaccinated animals in contrast with 2/4 unvaccinated animals. Flint and Liardet (1980) assessed the efficacy of a trivalent Hardjo, Pomona and Copenhageni vaccine to prevent urinary shedding in 20 four-six month old calves sero-negative to the serovars included in the vaccine (MAT <10). Calves were divided into an unvaccinated group (n=10) and a vaccinated group (n=10) that received two vaccine doses 21 days apart. The two groups were challenged intraperitoneally with Hardjo 14 days after the vaccinated group received the second dose. Despite several attempts, no leptospires were isolated from the urine of vaccinated calves compared to 7/10 unvaccinated calves found shedding leptospires in urine.

1.11.2 Deer and sheep

Despite that few studies have been conducted to evaluate the efficacy of vaccines to prevent shedding of leptospires in the urine of either deer or sheep, their results have shown that vaccines were able to prevent shedding of not previously exposed animals.

In deer, a split-herd study under natural Hardjo-bovis and Pomona challenge in

New Zealand was conducted by Ayanegui-Alcerreca (2006) who selected six herds in two years from three farms serologically positive to Hardjo-bovis or Pomona, and a fourth farm without evidence of infection. Fifty to seventy-five percent of the deer in each herd were given two doses, separated by a month, of a Hardjo, Pomona and Copenhageni vaccine. Two to five urine samples were taken from rising one year old females in a period that ranged from 5 to 12 months from first vaccination. Overall vaccine efficacy to prevent shedding of leptospires in urine as assessed by dark field microscopy (DFM) was estimated as 44%. These farms had serological evidence of *Leptospira* on the day of vaccination, which may have limited the vaccine effect since vaccination has little impact on reducing shedding in already infected animals. DFM is commonly regarded as an insensitive method to detect leptospires in urine, especially when low *Leptospira* numbers are present (Levett 2001). This suggests that i) vaccinated animals in this trial may have shed high numbers of leptospires in urine in order to be detected, and ii) the 44% vaccine efficacy observed in the study may have been even lower, if vaccinated animals shedding low numbers of leptospires in urine were regarded as negative by the test.

In another study, Subharat et al. (2012a) selected 220 three-months-old females from five farms for a vaccination trial. The animals received antibiotic treatment to eliminate any possible infection before vaccination. Vaccinated animals received two doses of a Hardjo and Pomona vaccine, 28 days apart. After seven months from the application of the second dose, no vaccinated deer had detectable leptospires by either PCR or culture in contrast to 5/9 and 3/25 antibiotic treated and unvaccinated deer that had PCR positive urine on two farms, respectively and 1/9 treated unvaccinated deer on one farm that had leptospires isolated from urine.

In sheep, Webster and Reynolds (1955) reported two vaccination trials conducted using either 16 or 40 recently weaned lambs. In each trial, lambs were divided in equal number into vaccinated and unvaccinated groups. About two to three weeks after the second dose of vaccine was given to vaccinated animals, lambs were challenged intraperitoneally with Pomona. No leptospires were observed in the urine of the two groups of vaccinated lambs as opposed to 3/8 and 16/20 unvaccinated lambs that had leptospires isolated from urine in each trial, respectively. Although significance was not tested in these trials, Fisher exact test p-values of reported frequencies suggest that vaccination significantly prevented urinary shedding in the larger trial ($p<0.001$). Marshall et al. (1979b) administered two doses, a month

apart, of a Hardjo-Pomona vaccine in seven nine-month-old sheep. Six weeks after the last dose, all vaccinated lambs and the 10 controls used were challenged intraperitoneally and intramuscularly with Hardjo. Hardjo was cultured from kidneys of 10/10 unvaccinated and 2/9 vaccinated sheep ($p<0.001$).

1.12 Multivalent and monovalent vaccines

It has been reported that multivalent vaccines containing serovar Hardjo are not able to provide the same level of protection against Hardjo challenge as monovalent Hardjo vaccines (Brown et al. 2003). Some efficacy studies observed that multivalent vaccines containing serovar Hardjo conferred poor protection against *Leptospira* shedding in urine of cattle after challenge with Hardjo (Bolin et al. 1989a; Bolin et al. 1989b). In contrast, a high level of protection (100%) against shedding was observed for Hardjo monovalent vaccines (Bolin and Alt 2001). The difference was later attributed to the strong cell-mediated immune response that the monovalent vaccine was able to trigger in contrast with a weaker cell-mediated immune response observed for the multivalent vaccine (Brown et al. 2003). Nevertheless, these findings did not provide conclusive evidence in favour of monovalent Hardjo vaccines since lack of protection against urinary shedding has also been observed in trials using monovalent Hardjo vaccines. For example, Plunkett et al. (2013) observed that vaccination with a Hardjo monovalent vaccine failed to prevent significantly shedding of leptospires in urine of cattle (assessed by FA) naturally exposed to Hardjo. On the other hand, multivalent vaccines were also shown to prevent shedding of leptospires in urine of vaccinated animals compared with unvaccinated controls as observed by Rinehart et al. (2012) who challenged sero-negative heifers with Hardjo, on three consecutive days, 105 days on from vaccination with a multivalent vaccine. No leptospires were isolated from urine of vaccinated heifers in contrast to 11/11 unvaccinated heifers from which leptospires were isolated from urine. In another study assessing the efficacy of a multivalent *Leptospira* vaccine, Zimmerman et al. (2013) observed that vaccination prevented shedding of leptospires in urine after Hardjo challenge, as observed by culture, in 4/18 vaccinated as opposed to 18/18 unvaccinated calves having Hardjo isolated from urine.

1.13 The long term efficacy of vaccination to prevent urinary shedding

Trials evaluating the long term efficacy of vaccination to prevent urinary shedding under natural challenge have been conducted. Their results suggest that vaccination reduces the numbers of animals shedding leptospires in urine compared to unvaccinated animals but in some cases isolations at a single point in time were made in vaccinated animals, suggesting transient infections. Mackintosh et al. (1980b) assessed the long-term efficacy of a Hardjo/Pomona vaccine in cattle. Despite observing no vaccinated animal and 9/10 unvaccinated controls shedding leptospires in urine at 56 weeks from first vaccination, transient isolations of *Leptospira* were made in 2/8 animals at one point in time for each (2/83 isolation attempts) compared with a more consistent pattern of isolations in unvaccinated animals (45/106 isolation attempts). A similar result was observed by Allen et al. (1982) who reported that one out of seven vaccinated and 13 out of 24 unvaccinated animals, initially found shedding leptospires in urine at 18 weeks after initial vaccination, were still shedding four weeks later. It may be possible that the lack of sensitivity of culture to detect leptospires in urine, especially in vaccinated animals due potentially to a lower number of leptospires shed in urine, explain these observations.

Broughton et al. (1984) vaccinated twice, six weeks apart, a group of three-four months old calves. They, and their unvaccinated counterparts, were followed for 52 weeks. At 37 weeks from vaccination, sero-conversion was observed in unvaccinated controls. At 52 weeks 10/10 controls and 2/9 vaccinated cattle had sero-converted due to natural challenge. Despite evidence of sero-conversion in vaccinated animals, no leptospires were isolated from urine in comparison to 10/10 unvaccinated controls that had leptospires isolated from urine. Similarly, Marshall et al. (1979a) followed sero-negative calves that were allocated either into a vaccinated or unvaccinated group at the age of three-four months for a period of 46 weeks. At about 31 weeks after second vaccination, natural infection occurred in control animals. By 46 weeks from vaccination, 2/10 vaccinated and 10/10 controls had sero-converted but no leptospires were isolated from urine of the vaccinated animals in contrast with 6/10 non-vaccinated calves that had *Leptospira* isolated from urine. The combined results initiated by Allen et al. (1982) and continued by Hancock et al. (1984) showed a reduction of the proportion of shedders (assessed by DFM and culture)

in the vaccinated group (n=37) from 17.9% at 18 weeks after calf-hood vaccination to 2.7% at about 55 weeks after calf-hood vaccination and prior to booster vaccination; compared to a 55.8% and 58.5% of unvaccinated shedders (n=41) detected at the same time points, respectively. At 77 weeks after calf-hood vaccination, heifers receiving two vaccine doses at 9-10 months of age only, or two vaccine doses at 9-10 months old plus a booster at 22-23 months of age had a lower percentage of animals shedding leptospires in urine (4/30) compared to the percentage of shedders (4/9) observed in unvaccinated heifers.

A *Leptospira* elimination programme, consisting of vaccination of the whole beef herd in a Scottish island, was conducted by Little et al. (1992a). Previously, antibiotic treatment and management changes were evaluated and soon discarded due to their inability to eliminate infection from the herd (Little et al. 1992b). The vaccination programme consisted of two initial vaccine doses, four to eight weeks apart and annually thereafter for five years of the whole herd. The overall prevalence of Hardjo titres across the age groups in the herd was reduced from 48.3% in the first year to 16.3% in the fifth year but in cattle from one to three years old, only one out of 233 had a positive Hardjo titre in the fifth year in contrast to 108 out of 320 cattle of the same age group sero-positive to Hardjo in the first year. No isolations were made in urine of 406 vaccinated cattle suggesting that the vaccination programme was successful in eliminating Hardjo infection from the herd.

A recent non-peer reviewed pilot study assessed the prevalence of urinary shedding of leptospires in dairy herds with a history of annual vaccination (Wilson et al. 2013). They found that 30% of herds and 13% of cows in positive herds had evidence of urinary shedding of leptospires by PCR. Cows were not tested serologically for the infecting serovar(s), thus *Leptospira* serovar was unknown. A research project currently looking into the long-term efficacy of vaccination in dairy cattle in New Zealand will provide more insights on this topic.

1.14 Conclusion

Leptospirosis notified cases have decreased in recent years. However, human leptospirosis in New Zealand continues at a high rate compared with other developed countries and affecting predominately people working in close contact with livestock.

Animal vaccination trials suggested that vaccination of naïve individual prevents later infection and shedding of leptospires in urine of cattle, sheep and deer in a moderate to high extent and therefore human exposure to *Leptospira* could be potentially reduced if vaccine coverage in livestock were increased. It is important to highlight the benefit of annual herd vaccination over individual animal protection to reduce *Leptospira* exposure in animals and humans since individual animal vaccination failures are expected to be overcome by herd immunity.

Production losses attributed to leptospirosis were more evident in deer than cattle or sheep. It is possible that these losses can be prevented by vaccination. Few reports have investigated the cost-benefit of annual vaccination in livestock. Since cost can represent a barrier for the adoption of this practice by the farmer, further research should address this in the future.

The method used to detect leptospires in urine is an important factor to take into account when assessing vaccine efficacy to prevent urinary shedding of leptospires. Although PCR is regarded as a highly sensitive and specific test compared with culture, the clinical and epidemiological importance of a positive PCR result should be further explored.

Despite evidence in favour of some Hardjo monovalent vaccines over multivalent vaccines to prevent shedding of leptospires in urine of cattle, additional attempts should be made to compare the preventive effect of monovalent and multivalent vaccines, particularly under farm conditions and natural challenge.

Finally, there is little evidence supporting that MDA against *Leptospira* interfere with the efficacy of leptospiral vaccines, although few robust studies appear in the literature. The question of timing of first vaccination and therefore impact of MDA is likely critical to the success of a vaccination programme. Individual variability of MDA duration in new-born animals, on farm conditions, and potential interference

with vaccination are areas that need to be investigated.

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Chapter 2

Prevalence and risk factors for *Leptospira* sero-positivity in New Zealand farmers

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2.1 Summary

Leptospirosis is a global zoonosis that in New Zealand primarily affects people occupationally exposed to livestock. The objective of this study was to estimate the sero-prevalence of five *Leptospira* serovars in farmers working on cattle, sheep, and deer farms that had the serological status of animals previously assessed; and to identify risk factors for farmer sero-positivity. A total of 178 farmers from 127 properties participated in the study. Blood samples were tested using the microscopic agglutination test (MAT) for presence of antibodies to *Leptospira*. Samples with a MAT titre $\geq$48 were considered sero-positive. Using Bayesian statistical analysis, the median sero-prevalence of *Leptospira*, all serovars combined, was estimated to be 6.6% (95% probability interval (PI) 3.6%–10.9%). Risk factors associated with sero-positivity were assisting deer or cattle calving, farming deer, having $\geq$25% of flat terrain, and high abundance of wild deer on farm, whilst high possum abundance on farm was negatively associated with sero-positivity. No association was observed between farmer sero-status and previously recorded livestock serology. *Leptospira* sero-positivity was associated with influenza-like illness of farmers (RR=1.7; 95% PI 1.0–2.5). Assuming a causal relationship, this suggested an annual risk of 1.3% (PI 0.0%–3.0%) of influenza-like illnesses due to *Leptospira* infection in the population of farmers. The association between sero-positivity and disease can be used to estimate the public health burden of leptospirosis in New Zealand. Identifying and understanding risk factors for *Leptospira* sero-positivity can inform preventive measures, hence contributing to reduction of the incidence of leptospirosis in farmers.

2.2 Introduction

Leptospirosis is a zoonosis that affects a wide range of mammal species. Once infected, animals can carry leptospires in renal tissue for variable periods of time, excreting them through urine into the environment. Humans usually become infected by contact with the urine of an infected animal or with a urine-contaminated environment. Mucosal and conjunctival tissues and scratches or cuts in the skin are common entry routes for leptospires (Levett 2001; Adler and De la Peña Moctezuma 2010). Infection in humans can result in a severe, potentially fatal illness but in most cases it remains asymptomatic or generates mild disease. Signs and symptoms of disease are non-specific but may include fever, headache, myalgia, dry cough, abdominal pain, and nausea (Levett 2001; Haake and Levett 2015). Thus, leptospirosis may be misdiagnosed as influenza or other febrile illness.

From 2001 to 2014, there were on average 91 notified human cases per year in New Zealand, equivalent to 2.2 cases per 100,000 people annually. Farmers and abattoir workers represented on average 80% of notified cases during this period (ESR 2002-2015). Historically, *Leptospira borgpetersenii* serovar Hardjo (Hardjo-bovis) and *Leptospira interrogans* serovar Pomona (Pomona) were the two most commonly reported serovars in notified cases. However, during the 1990s the incidence of *Leptospira borgpetersenii* serovar Ballum (Ballum) increased (Thornley et al. 2002) and Ballum was the most common serovar identified in human notified cases in 2010-11 (ESR 2002-2015).

Serological surveys in dry-stock showed that *Leptospira* sero-positivity is common with mean estimates ranging from 44% to 50% for Hardjo-bovis and from 11% to 25% for Pomona in beef cattle, from 20% to 43% for Hardjo-bovis and from 3% to 14% for Pomona in sheep, and from 25% to 26% for Hardjo-bovis and from 8% to 11% for Pomona in deer (Ayanegui-Alcerreca et al. 2010; Dreyfus et al. 2011; Subharat et al. 2012). Incidental infections with Ballum and *Leptospira interrogans* serovar Copenhageni (Copenhageni) have been observed in cattle (Ris et al. 1973) and serological evidence of Ballum, Copenhageni, and *Leptospira borgpetersenii* serovar Tarassovi (Tarassovi) has been reported in farmed deer (Flint et al. 1988; Wilson et al. 1998; Ayanegui-Alcerreca et al. 2010).

The relatively high sero-prevalence of Hardjo-bovis and Pomona in dry-stock

may result in high levels of urinary shedding. Fang et al. (2015) showed that 41% of sero-positive cattle and sheep (MAT $\geq$48) to Hardjo-bovis or Pomona were shedding leptospires in urine (PCR positive), potentially exposing people in close contact with these animals. In a sheep only abattoir it was observed that 13/1,000 sheep were kidney culture positive for *Leptospira*; hence potentially excreting the organism in urine (Dorjee et al. 2008). In view of the large numbers of sheep processed in a day, it was estimated that each worker in the abattoir studied was exposed to 3-54 infected sheep depending on the work position and the time of the year (Dorjee et al. 2011). Dreyfus et al. (2014) observed that 10.9% of workers in eight abattoirs processing sheep, cattle or deer were sero-positive to either Hardjo-bovis or Pomona.

In veterinarians, an occupational group scarcely represented among notified cases, a serological survey estimated sero-prevalence to be 5.1% for Hardjo-bovis, Pomona, Ballum, Copenhageni or Tarassovi (Sanhueza et al. 2015). In farmers, Blackmore and Schollum (1982) observed 6/76 (7.9%) sero-positive beef and sheep farmers of the North Island of New Zealand. However, there is no recent estimate of the sero-prevalence or factors associated with sero-positivity of farmers, despite being frequently reported among notified cases (ESR 2002-2015).

In New Zealand, about 90% of dairy cattle are vaccinated annually against leptospirosis but vaccination is far less frequently used in beef cattle (mean range=18%–25%) or deer (mean range=5%–9%), and almost never in sheep (mean<1%) (Wilson et al. 2008; Heuer 2009; Dreyfus et al. 2011). Although vaccination of animals is believed to be the most effective tool for reducing human infection (Marshall, 1987), the cost-benefit of vaccination may represent a limitation for widespread adoption of this practice in dry-stock farming. Therefore, identifying and managing risk factors for human sero-positivity on dry-stock farms may contribute to reduction of the incidence of leptospirosis in farmers. The aims of this study were to determine the sero-prevalence of Hardjo-bovis, Pomona, Ballum, Copenhageni and Tarassovi in beef cattle, sheep, and/or deer farmers; and to identify risk factors for *Leptospira* sero-positivity in this occupational group.

2.3 Material and Methods

2.3.1 Sampling frame

The sampling frame was 238 commercial farms in an earlier study, selected in a stratified-random fashion from 1,940 respondents to a mail survey, who consented to blood-sampling and testing of their animals for antibodies against *Leptospira* (20 per farm and species) between June 2009 and July 2010. Results have been reported elsewhere (Dreyfus et al. 2011). Those farmers were contacted again between December 2012 and February 2013 for participation in this study. Additionally, three farms that had the serological status of their livestock assessed in 2012 (E. Vallée personal communication, 2013), agreed to take part in this cross-sectional study. The serological status of animals was compared with serological results of farmers from the same farm.

2.3.2 Sample collection

Two certified phlebotomists were contracted for visiting each farm. A blood sample was taken from farmers and farm workers willing to participate between May and October 2013. Samples were chilled and transported to the Massey University leptospirosis laboratory where serum was separated from clotted blood and frozen within 24 hours of collection. Diluted sera (1:6) were stored in master plates at -18°C until testing.

2.3.3 Serological testing

Antibodies to *Leptospira borgpetersenii* serovar Hardjo (Hardjo-bovis), *Leptospira interrogans* serovar Pomona (Pomona), *Leptospira borgpetersenii* serovar Ballum (Ballum), *Leptospira borgpetersenii* serovar Tarassovi (Tarassovi), and *Leptospira interrogans* serovar Copenhageni (Copenhageni) were tested in the mEpiLab[1], Hopkirk Institute, Massey University. A modified microscopic agglutination test (MAT) based on the methodology described by Faine et al. (1999) was used. In summary, eight serial doubling dilutions of serum using 0.9% saline solution were prepared. Antigen was then added to obtain a set of serum dilutions from 1:24 to 1:3072. The

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final MAT titre was the highest dilution able to agglutinate at least 50% of antigen. A reciprocal titre of 1:48 or higher was regarded as sero-positive for the statistical analysis and interpreted as previous infection. For description purposes, all MAT titres are presented.

2.3.4 Questionnaire

A questionnaire was designed to record potential risk factors for *Leptospira* sero-status. This included personal information, activities performed within the farm, activities performed outside the farm, previous leptospirosis episodes recalled by farmers at any moment during their life, influenza-like illness episodes in the 18 months prior to the survey, and information about the farm environment such as terrain, wildlife abundance and flooding (Appendix I: Farmer questionnaire). The questionnaire was completed by each participant at the time of blood sampling.

2.3.5 Data description

Age and gender of participating farmers was described using summary statistics. The percentage of farmers exposed to beef cattle, sheep, and deer; and the percentage of farms vaccinating their animals against leptospirosis, was also described.

Since a farm could contribute more than one person to the sample, a farm was considered sero-positive when at least one farmer/farm worker was sero-positive to *Leptospira* at the MAT titre cut point of $\geq$48.

2.3.6 Sero-prevalence in farmers and animals

A Bayesian intercept-only logistic regression model was developed to estimate farmer sero-prevalence for Hardjo-bovis, Pomona, Ballum, Tarassovi, and/or Copenhageni; and livestock sero-prevalence for Hardjo, and/or Pomona from the same farms using a subset of previously recorded animal data (Dreyfus et al. 2011). In each model, the posterior distribution of the sero-prevalence was estimated using the formula: prevalence=$\frac{e^{\beta_0}}{1+e^{\beta_0}}$ where β_0 is the intercept of the logistic model. The median of the posterior distribution and 95% probability interval (95% PI) are pre-

sented. A diffuse prior of $mean = 0$ and $precision = 0.0001$ ($\mathcal{N}(0, 0.0001)$) was used for the intercept at the logit scale (Appendix II: Prevalence model). For animal sero-prevalence estimation, a random effect was added to the model to account for the clustering of animals within herds/flocks (Appendix III: Prevalence model clustering). For farmer sero-prevalence estimation, no adjustment for the cluster effect was required since the intra-cluster correlation (ICC) of farmer's sero-status within each farm was negligible ($ICC = 9.84e^{-8}$).

2.3.7 Multivariable model building

Unadjusted associations between *Leptospira* sero-status (MAT titre $\geq$48) of farmers and putative risk factors were initially screened using a Bayesian logistic regression model (Appendix IV: Multivariable model). A diffuse prior ($\mathcal{N}(mean = 0, precision = 0.0001)$) was used for each coefficient. Variables with an associated proportion of the posterior distribution crossing the value of zero $\leq$0.2 were selected for inclusion in the multivariable model. Backward elimination of variables was guided by the proportion of the posterior distribution changing the sign of the median value and based on the deviance information criterion (DIC) (Spiegelhalter et al. 2002).

2.3.8 Multivariable model specification and convergence diagnostics

An initial burn-in of 5,000 iterations was followed by 30,000 iterations used for estimation of coefficients. Prevalence ratio (PR) and 95% PI were reported in the final multivariable model. Markov Chain Monte Carlo (MCMC) convergence was assessed visually using traceplots of three chains and autocorrelation plots, and statistically using Gelman and Rubin diagnostics (Gelman and Rubin 1992).

2.3.9 Leptospirosis in farmers pre-sampling

Information about previously confirmed leptospirosis episodes that occurred at any time during the farmer's life-time, as recalled by participants, was recorded. This

included the year of illness, symptoms, number of days off-work, and whether hospitalization was required. The association between previous leptospirosis episodes (yes/no) and sero-status (sero-positive/sero-negative) was evaluated. The variable "previous leptospirosis episodes" was excluded from the multivariable model of present sero-status as it could mask occupational or behavioural risk factors associated with *Leptospira* sero-positivity.

2.3.10 Influenza-like illness

The occurrence of influenza-like illness in the 18 months preceding the date of sampling was recorded. The disease was defined as an episode of illness accompanied by any of the following signs or symptoms: fever, headache, myalgia, photophobia, sweating, and general debility. Assuming a causal association between *Leptospira* sero-positivity and the occurrence of influenza-like illness, PR, population attributable risk (PAR) and population attributable fraction (PAF) were estimated using a multinomial Bayesian model (Pirikahu et al. 2016).

2.3.11 Statistical software

Analyses were performed using R version 3.1.2 (R Core Team 2015) and JAGS (Plummer 2003).

2.3.12 Human ethics application

Ethical approval was obtained from the Massey University Human Ethics Committee (MUHEC: Southern A Application - 12/10).

2.4 Results

People from 127 farms agreed to participate in the study (78 in the North Island and 49 in the South Island). Blood samples were collected from one farmer in 91 farms, two in 25 farms, three in seven farms, and four in four farms (Total = 178). The average age of participants was 53 years (Min=20, Max=76 years), and 159 were male. The spatial point location of contacted farms, participant farms and farms with sero-positive and sero-negative farmers is presented in Figure 2.1. Sero-positive farms were present in most regions sampled, five were from Hawke's Bay (a region that had the highest number of notified cases during 2013 (ESR 2014)). The sero-prevalence in this region was 4.0 times (95% PI 1.4–10.5) higher than that of other regions.

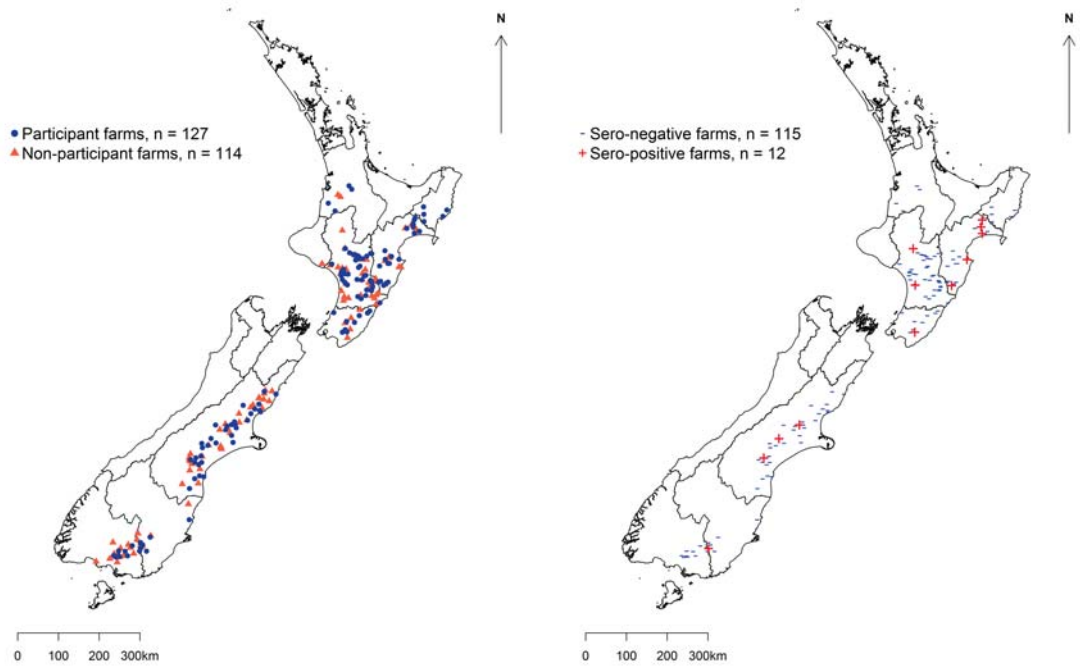

Figure 2.1: Spatial point location of participant and non-participant farms (left), and farms with *Leptospira* sero-positive and sero-negative farmers (right).

2.4.1 Serology in farmers

The frequency of MAT titres by serovar in farmers is shown in Figure 2.2.

Twelve farmers (6.6%, 95% PI 3.6%–10.9%) were sero-positive to any of the

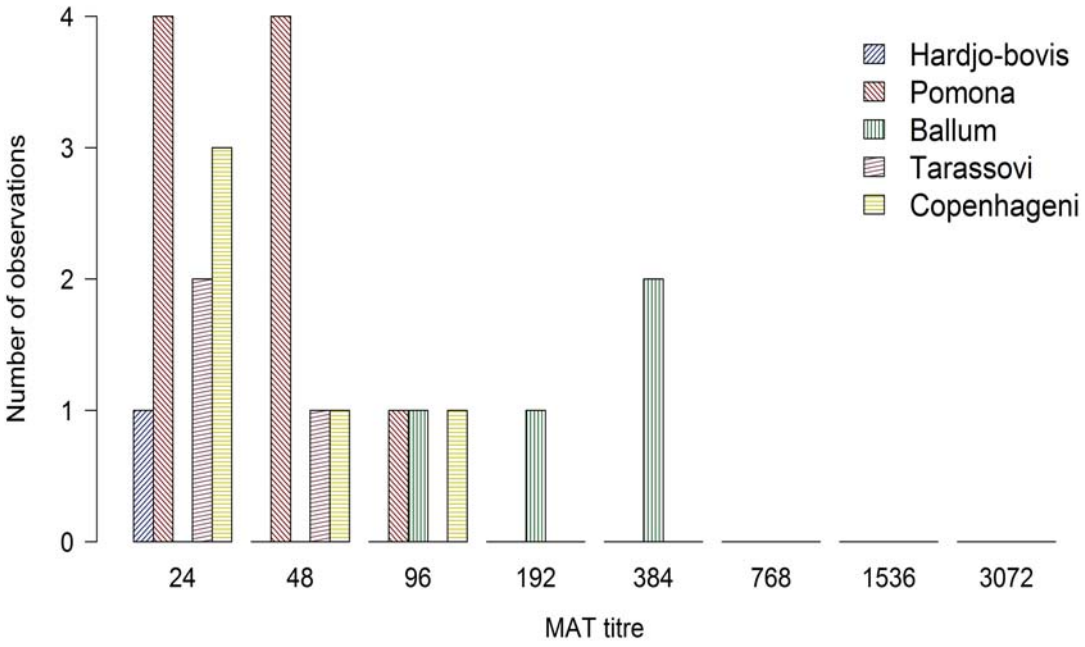

Figure 2.2: MAT antibody titre distribution for each *Leptospira* serovar in farmers.

Leptospira serovars tested. The most prevalent serovars were Pomona (2.6%) and Ballum (2.1%), followed by Copenhageni (1.0%) and Tarassovi (0.4%). Titres for Ballum ranged from 96 to 384 while for Pomona and Copenhageni titres ranged from 24 to 96. No farmer had a MAT titre ≥48 to Hardjo-bovis (Table 2.1). All sero-positive farmers were male and each came from a different farm; hence the farm level prevalence was 9.5% (95% PI 5.3%–15.5%).

Table 2.1: Sero-prevalence (%, MAT titre ≥48) of farmers (n=178) and 95% probability interval (95% PI) stratified by serovar and positive to at least one serovar (overall).

Serovar	**n positive**	**Mean %**	**Median % (95% PI)**
Pomona	5	2.8	2.6 (0.9–5.7)
Ballum	4	2.3	2.1 (0.6–4.9)
Copenhageni	2	1.1	1.0 (0.1–3.1)
Tarassovi	1	0.6	0.4 (0.0–2.1)
Hardjo-bovis	0	0.0	0.0 (0.0–0.0)
Overall	12	6.8	6.6 (3.6–10.9)

2.4.2 Animal contact, vaccination and previous animal serological status

The animal and herd serological status of the 238 farms sampled in 2009-10 was reported by Dreyfus et al. (2011). Here we present animal serological results of the subset of 124 farms plus the three additional farms (E. Vallée, personal communication, 2013) that had farmer sero-status assessed in the present study.

Twelve farmers were in contact with a single animal species (two farmers with beef cattle only, six farmers with sheep only, and four farmers with deer only). All other 166 farmers had contact with two or more animal species (92 farmers with beef and sheep, five farmers with beef and deer, 14 farmers with sheep and deer, and 55 farmers with beef and sheep and deer). Overall, 100 participants farmed beef and/or sheep without deer contact, and 78 farmed deer alone or in combination with beef and/or sheep. Animals were vaccinated against *Leptospira* in 27/64 (42.2%) of beef cattle herds, 7/54 (13.0%) of deer herds and 1/86 (1.2%) of sheep flocks.

Table 2.2 shows the sero-prevalence for Hardjo-bovis and Pomona at the animal and herd level for the species sampled. At the animal level, sero-positivity to Hardjo-bovis was frequently observed in beef cattle (52.2%) and sheep (43.1%) and to a lesser extent in deer (6.4%). Pomona sero-positivity was less frequent than Hardjo-bovis sero-positivity in these species although, evidence of Pomona sero-positivity was observed in more than half of the herds for any species. On farms with at least one sero-positive animal, Hardjo-bovis and Pomona were slightly more prevalent in beef cattle, than in sheep or deer.

2.4.3 Association between previous animal sero-prevalence and farmer sero-status

The cluster adjusted animal sero-prevalence for Pomona on farms having a sero-positive farmer was estimated to be 7.0% (95% PI 1.4%–20.9%), which was similar to the 10.8% (95% PI 8.3%–13.7%) Pomona sero-prevalence estimated on farms with no farmer sero-positive to this serovar. Neither Hardjo-bovis nor Pomona sero-prevalence of livestock were associated with *Leptospira* sero-positivity of farmers.

Table 2.2: Median herd- and animal-level sero-prevalence (%) and 95% probability interval (95% PI) for Hardjo-bovis and Pomona in livestock from 127[a] farms. Animal level sero-prevalence is shown for all herds/flocks and in herds/flocks with at least one sero-positive animal.

		Sero-prevalence % (95% PI)				
Animal level	**n**	**Hardjo-bovis**	**n**	**Pomona**	**n**	**Hardjo-bovis/Pomona**
Beef cattle all herds	1374	52.2 (42.3–62.1)	1374	17.7 (10.8–26.6)	1374	65.3 (54.8–74.7)
Beef cattle +ve herds	1293	57.4 (47.0–67.0)	1107	31.0 (22.1–41.0)	1354	66.3 (56.5–75.3)
Sheep all flocks	2178	43.1 (32.7–54.0)	2178	8.7 (6.2–11.4)	2178	54.3 (44.5–63.4)
Sheep +ve flocks	2012	51.5 (42.0–61.0)	1726	14.5 (11.4–17.9)	2118	57.0 (48.1–65.7)
Deer all herds	1133	6.4 (2.1–15.0)	1133	4.2 (2.1–7.2)	1133	20.2 (10.9–32.8)
Deer +ve herds	637	41.6 (27.3–56.8)	639	14.1 (9.6–19.2)	845	42.1 (30.9–54.3)
Herd level						
Beef cattle	66	93.9 (85.4–97.6)	66	78.8 (67.5–86.9)	66	98.5 (91.9–99.7)
Sheep	92	91.3 (83.8–95.5)	92	76.1 (66.4–83.6)	92	96.7 (90.8–98.9)
Deer	56	57.1 (44.1–69.2)	56	57.1 (44.1–69.2)	56	75.0 (62.3–84.5)

[a] Subset of 124 farm results from Dreyfus et al. (2011) and the three additional farms (E. Vallée, personal communication, 2013).

2.4.4 Risk factors for *Leptospira* sero-positivity in farmers

The unconditional association between *Leptospira* sero-status and 61 putative risk factors was initially assessed for inclusion in the multivariable model. Twenty potential risk factors that were liberally associated with *Leptospira* sero-status are shown in Table 2.3.

Table 2.3: Unconditional association, and 95% probability interval (95% PI), between *Leptospira* sero-status and 20 potential risk factors. The proportion of the posterior distribution for each coefficient that was below or above 0 (p) is shown. Only potential risk factors with p $\leq$0.2 are presented.

Risk factor	**Category**	**n**	**Coefficient (95% PI)**	**p**
Smoking at work	Yes	9	1.48 (⁻0.75–3.10)	0.07
	No	169		
Assisting calving/fawning	Yes	87	1.24 (⁻0.07–2.86)	0.03
	No	91		
Pet dog in house	Yes	107	1.29 (⁻0.16–3.36)	0.05
	No	70		
Cats in house	Yes	123	0.95 (⁻0.50–2.97)	0.13
	No	55		
Home slaughter	Yes	154	0.94 (⁻0.94–4.91)	0.20
	No	25		
Home slaughter beef	Yes	41	⁻0.73 (⁻2.29–0.55)	0.13
	No	137		
Home slaughter sheep	Yes	76	1.52 (⁻0.31–4.28)	0.08
	No	102		
Wild deer abundance on farm	High	27	1.53 (0.21–2.83)	0.02
	Low	151		
Rabbit abundance on farm	High	85	⁻1.22 (⁻2.81–0.12)	0.04
	Low	92		
Possum abundance on farm	High	48	⁻1.94 (⁻6.04–⁻0.03)	0.02
	Low	130		
Hay fever	Yes	38	0.62 (⁻0.70–1.92)	0.11
	No	140		
Valley pond water source	Yes	94	⁻1.46 (⁻3.08–⁻0.15)	0.02
	No	79		
Farming dairy	Yes	49	0.65 (⁻0.65–1.86)	0.14

	No	129		
Species farmed	DSB[a]	78	1.06 (⁻0.12–2.52)	0.03
	SB[b]	100		
Farming goat	Yes	170	⁻2.33 (⁻3.96–⁻0.59)	0.01
	No	8		
Flat terrain on farm	≥25%	90	0.76 (⁻0.46–2.14)	0.11
	<25%	88		
Beef number on farm×100	Continuous	162	⁻0.09 (⁻0.31–0.06)	0.12
Sheep number on farm×100	Continuous	167	⁻0.03 (⁻0.06–⁻0.01)	0.01
Pomona prevalence beef×100	Continuous	99	⁻1.78 (⁻7.12–1.84)	0.19
Pomona prevalence deer×100	Continuous	76	⁻3.02 (⁻11.78–2.63)	0.17

[a] Deer alone or with beef and/or sheep.
[b] Beef and/or sheep.

The full multivariable model included the 20 potential risk factors shown in Table 2.3. The final multivariable model included the complete set of data from 178 farmers. Factors associated with *Leptospira* exposure and PR are summarised in Table 2.4. Farmers had a higher sero-prevalence when they assisted either cattle or deer calving (PR=7.2), worked on farms with high abundance of wild deer (PR=10.8), farmed deer (PR=6.9) or worked on farms where at least 25% of the total farm land was flat terrain (PR=4.2). Possum abundance was associated with a decreased sero-prevalence of *Leptospira* in farmers.

2.4.5 Previous leptospirosis

Ten farmers (6%) recalled being diagnosed with leptospirosis at some time during their lives. They reported being seriously ill for a median of 10 days. Two farmers reported being ill for more than six months and four required hospitalisation. No information about the infecting serovar was available. The most common signs/symptoms were fever (10/10), sweating (10/10), myalgia (10/10), severe headaches (9/10), sore eyes (7/10), photophobia (6/10), severe debility (4/10), jaundice (1/10), and renal and hepatic malfunction (1/10). Eight leptospirosis episodes occurred between two and 36 years before sampling, and two episodes had occurred the same year of sampling. Farmers previously diagnosed with leptospirosis were 10.9 (95% PI 4.2–27.2) times as likely to be sero-positive as farmers not diagnosed with leptospirosis in the past. The association remained strong (PR=8.5; 95% PI

Table 2.4: Results of the final multivariable Bayesian logistic regression model showing the association between *Leptospira* sero-status and risk factors. Number of observations (n), coefficients, median prevalence ratio (PR) and 95% probability interval (95% PI) are presented.

Risk factor	**Levels**	**n**	**Coefficient**	**PR**	**95% PI**
Intercept	-		$^{-}$6.39		
Assisting calving/fawning	Yes	87	2.02	7.2	1.7–42.7
	No	91	Ref.		
Wild deer abundance on farm	High	27	2.52	10.8	2.4–57.0
	Low	151	Ref.		
Species farmed	DSB[a]	78	1.99	6.9	1.6–40.8
	SB[b]	100	Ref.		
Flat terrain on farm	≥25%	90	1.47	4.2	1.1–20.9
	<25%	88	Ref.		
Possum abundance on farm	High	48	$^{-}$2.42	0.1	0.0–0.7
	Low	130	Ref.		

[a] Deer alone or with beef and/or sheep.
[b] Beef and/or sheep.

2.6–23.1) even when the two episodes that occurred during 2013, the year of sampling, were excluded.

2.4.6 Association between serology and influenza-like illness

Seventy participants suffered from influenza-like illness in the 18 months prior to sampling. Farmers that were sero-positive to *Leptospira* had 1.7 (95% PI 1.0–2.5) times greater risk of reporting influenza-like illness during that period than farmers sero-negative to *Leptospira*. It was estimated that the annual incidence of influenza-like illnesses attributed to *Leptospira* exposure was 1.3%. Population attributable risk and fraction estimates presented in Table 2.5 were scaled to reflect the risk of disease attributed to *Leptospira* exposure in a 12 month period.

Table 2.5: Association between *Leptospira* sero-status and influenza-like illness. Population attributable risk and fraction were adjusted for a period of 12 months instead of 18 months.

Sero-status	**ILI[a](+)**	**ILI[a](-)**	**RR[b] (95% PI)**	**PAR[c] % (95% PI)**	**PAF[d] % (95% PI)**
Leptospira (+)	8	4	1.7 (1.0–2.5)	1.3 (0.0–3.0)	3.4 (0.0–7.8)
Leptospira (-)	62	104			
Total	70	108			

[a]Influenza-like illness.
[b]Median risk ratio and 95% probability interval.
[c]Median population attributable risk % and 95% probability interval.
[d]Median population attributable fraction % and 95% probability interval.

2.5 Discussion

The sero-prevalence of *Leptospira* in farmers for any of the five serovars tested was estimated to be 6.6%. This estimate is higher than the the 0% observed in veterinary students (Fang et al. 2014) and slightly higher than the 5.1% observed in New Zealand veterinarians for the same serovars (Sanhueza et al. 2015) but lower than the 10.9% observed in New Zealand abattoir workers for Hardjo-bovis and/or Pomona (Dreyfus et al. 2014). While 2.6% of farmers were sero-positive for Pomona, none were sero-positive for Hardjo-bovis; a serovar which was endemic in sheep and beef cattle, being about 2.5-fold as frequent as Pomona in these species.

In the year that farmers were blood sampled (2013), a drought affected the country and there was a lower incidence of notified human leptospirosis cases (1.3 per 100,000) than in the preceding year (2.4 per 100,000) (ESR 2014). One may speculate that a drought could have limited *Leptospira* exposure of farmers, potentially decreasing the observed sero-prevalence, especially for serovars with comparatively shorter titre duration, such as Pomona (Dreyfus et al. 2015). This would be particularly so if it was shown that the duration of Pomona shedding in livestock was shorter than that for Hardjo-bovis.

Since it has been observed that the risk of having a PCR positive kidney or urine sample to *Leptospira* was higher in sero-positive animals (21%-41%) compared to sero-negative animals (1%-3%) (Dorjee et al. 2008; Fang et al. 2015), it was expected that a higher animal sero-prevalence on a farm, and consequently a higher proportion of shedders, would have subjected farmers working on that farm to a higher level of exposure, increasing sero-positivity. However, there was no correlation between serological status of farmers and animal sero-prevalence of Hardjo-bovis or Pomona. Although different routines or activities performed by farmers may modify the risk of infection, it is likely that the lack of association was due to the low power for contrasting animal sero-prevalence results associated with 12 sero-positive farmers and 166 sero-negative farmers. For this number of sero-positive and sero-negative farmers we would have detected only a difference of about 35% in animal sero-prevalence 80% of the time with a confidence level of 95%. To be considered is that the animal sero-prevalence data presented in Table 2.2 were obtained in 2009/10, whereas farmers were sampled in 2013. It has been shown that the serological status of beef, sheep and deer herds/flocks can change over consecutive years (Subharat et

al. 2012). Therefore, it is likely that the animal sero-prevalence and the proportion of shedders changed on some farms between 2009-10 and 2013 affecting a potential association between animal and farmer *Leptospira* sero-status.

A single elevated MAT titre (e.g. ≥800) and clinical symptoms of leptospirosis can be indicative of acute infection in humans (Levett 2001) but there is not a widely accepted defined titre cut-off for MAT positive sample when assessing past exposure in apparently healthy individuals. We chose a titre cut-off of ≥1:48 to detect past infection, not to diagnose acute leptospirosis. The results reported here can be directly compared with those previously reported in veterinarians, veterinary students and abattoir workers of New Zealand (Dreyfus et al. 2014; Fang et al. 2014; Sanhueza et al. 2015) since the same cut-off was used in those studies.

Antibody titres in humans may persist for an extended period of time after infection (Blackmore et al. 1984) depending, among other factors, on the infecting serogroup (Cumberland et al. 2001). Based on prevalence and incidence data from abattoir workers, it was estimated that MAT titres ≥48 last on average for 29 months or 10 months after Hardjo-bovis or Pomona infection, respectively (Dreyfus et al. 2015). In our study, two of the 10 previous leptospirosis episodes recalled by farmers occurred one to five months before sampling. Those farmers were sero-positive with titres of 96 for Pomona or 192 for Ballum. All other leptospirosis episodes occurred two to 36 years before sampling and yet a strong association (PR=8.5) between current sero-status and previous leptospirosis was observed, suggesting that antibody titres following clinical leptospirosis may last for many years, or re-exposure had occurred, i.e. farmers exposed in the past may be more likely to be re-exposed than farmers not exposed in the past, either due to behavioural/occupational activities or environmental factors that may modify *Leptospira* exposure. For instance, working on farms with ≥25% (median value) of flat terrain was associated with a higher sero-prevalence than working on farms with a different topography (<25% flat terrain). Leptospires can survive in the environment for variable periods of time (one to seven weeks) depending on the serovar and environmental conditions of temperature, humidity and pH (Hellstrom and Marshall 1978; Khairani-Bejo et al. 2004). Possibly, flat terrain is more prone to standing water puddles, which may increase *Leptospira* survival in the environment and consequently the opportunities for exposure of both animals and humans. Since topography is permanent, human re-exposure may be likely to occur more often on flat terrain than on farms with

predominantly steep slopes. However, dams are often used for stock water in hill country farms and arguably would also present a risk.

Farming deer alone, or in combination with beef and/or sheep, compared with not farming deer, increased the risk for *Leptospira* sero-positivity, which seems counter-intuitive since deer (animals and herds) showed the lowest sero-prevalence to either Hardjo-bovis or Pomona. However, deer are more volatile and unpredictable in yards, which usually have concrete floors where urine can be easily accumulated and splashed during animal movement. Further, deer often void dribbles of urine while being handled by farmers, who tend to have closer contact with deer than with cattle, and deer have a greater height than sheep, lessening the risk of exposure from the latter. Hence, it is plausible that a combination of behaviour, management practices and yard design contribute to deer farmers being at higher risk of *Leptospira* exposure than farmers of cattle and sheep (P.R. Wilson, personal communication, 2016).

Abortion is a rare event that has been associated with Pomona or Hardjo-bovis sero-positivity in cattle (Ellis et al. 1982; Sanhueza et al. 2013). Whether abortion occurs may depend on factors such as the infecting serovar and the age of the foetus at the time of infection (Elder et al. 1985; Ellis et al. 1986). Nonetheless, the majority of animals infected during pregnancy will deliver a calf while potentially shedding leptospires in urine. Bolin et al. (1989) observed that three out of four unvaccinated cattle, experimentally challenged with Hardjo-bovis during pregnancy, delivered apparently healthy calves carrying leptospires in the kidneys. The absence of antibodies in some calves infected *in utero* has raised the hypothesis of foetal immune tolerance to infection (Johnson et al. 1974; Bolin et al. 1989). The observation of an association between assisting cattle and deer calving, and *Leptospira* sero-positivity is plausible given the close contact during calving between a farmer and urine of potentially infected animals.

Leptospira can infect a wide range of mammals, including free-living species. In New Zealand, rats, mice, and hedgehogs were previously identified as reservoirs for serovar Ballum, whereas possums were mostly associated with *Leptospira borgpetersenii* serovar Balcanica infection (Hathaway and Blackmore 1981; Hathaway et al. 1981). Serological evidence of Pomona was observed in feral deer (Daniel 1966; Inglis 1984), although other serological surveys did not observe sero-positive wild

deer (Hathaway et al. 1981; Hathaway and Blackmore 1981). In our study, high wild deer abundance on farm was positively associated with farmer sero-positivity. Although it seems likely that wild deer can be infected and shed leptospires into the environment, the frequency of infection in the population and their role on human infection are not clear. Abundance of other free-living species as mice, rats, hedgehogs and possums on farm were not risk factors for *Leptospira* sero-positivity. Instead, high possum abundance was negatively associated with *Leptospira* sero-positivity. It is possible that this association was spurious by being a consequence of an unobserved factor causing both, *Leptospira* sero-positivity and possum abundance. For example, flat terrain increased the infection risk for farmers and it might reduce possum abundance due to lack of a typical habitat. On the other hand, steep slopes are more likely to be found around bush that favours possum abundance, while it would also reduce the exposure of farmers to standing water puddles. However, more comprehensive geographical, climate and vegetation data would be required to better understand these associations.

Leptospirosis in humans can range from a severe potentially fatal disease to a mild self-limiting disease that resembles influenza (Vickery et al. 2006; Haake and Levett 2015). An association (RR=1.7, 95% PI 1.0–2.5) between *Leptospira* sero-status and reported signs of influenza-like illness within 18 months prior to testing was observed. This finding is consistent with recent findings in abattoir workers (RR=1.9) and veterinarians (RR=1.4) (Dreyfus et al. 2015; Sanhueza et al. 2015). If we assume that *Leptospira* sero-conversion preceded and caused influenza-like illness, our findings suggest that 3.4% of influenza-like illnesses in a year may be attributable to *Leptospira* infection in the population of farmers in New Zealand (PAF). The corresponding annual risk of illness due to *Leptospira* infection was 1.3% (PAR), meaning that every year 13 in 1000 farmers would be exposed to *Leptospira* and develop clinical signs. This disease risk was lower than the 2.7% reported in abattoir workers but higher than the 0.5% reported in veterinarians in a year (Dreyfus et al. 2015; Sanhueza et al. 2015). These estimates of disease incidence attributed to *Leptospira* infection can inform models aiming to quantify the burden of leptospirosis in the country.

It may be possible that farmers who suffered from leptospirosis at some point in their life recalled episodes of influenza-like illness better than farmers who did not suffer from leptospirosis in the past. These clinical illness episodes occurred

2-36 years prior to the study and it is unlikely (though currently unknown) that these participants were still sero-positive. On the other hand, a recall bias could be present if farmers with previous leptospirosis associated any influenza-like signs with leptospirosis to a greater extent than farmers without previous leptospirosis, and they were also more likely to be sero-positive due to repeated exposure in risk-prone environments. However, such recall bias must have been small if present at all because there were only 10 of 178 farmers who reported a serious clinical episode of confirmed leptospirosis. Moreover, the leptospirosis awareness was similar in farmers with and without previous clinical leptospirosis (RR=1.1), and was not significantly associated with a recall of influenza-like disease or with sero-prevalence. We therefore conclude that recall bias did not impact on the inferences from this study and believe that sero-positive farmers were more likely to have had influenza-like illness than sero-negative farmers.

2.6 Conclusion

Leptospira sero-positivity was observed in 6.6% of currently healthy farmers of beef cattle, sheep and deer. The estimated sero-prevalence was similar to that of veterinarians and about half of that estimated for abattoir workers in New Zealand. Assisting in calving of beef cattle or deer, farming deer, farming on flat terrain, or with a high abundance of wild deer around the farm increased the risk of *Leptospira* sero-positivity. The association between *Leptospira* sero-status and influenza-like illness suggested that 13 of 1000 of these farmers may experience illness due to *Leptospira* infection every year, and that 3.4% of influenza-like illness in farmers may be attributable to *Leptospira* infection. These findings inform population estimates of the public health burden of leptospirosis in New Zealand and further our understanding of the epidemiology of *Leptospira* infection in the country.

2.7 Acknowledgement

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Chapter 3

Prevalence and risk factors for *Leptospira* sero-positivity in New Zealand veterinarians

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3.1 Summary

This study assessed sero-prevalence and risk factors for *Leptospira* (serovars Hardjo, Pomona, Ballum, Copenhageni, Tarassovi) sero-positivity in New Zealand veterinarians. Veterinarians (n=277) at one of two conferences were voluntarily enrolled and blood sampled. Microscopic Agglutination Test (MAT) titres $\geq$48 were considered sero-positive. Fourteen veterinarians (5.1%; 95% CI 2.8%–8.3%) were sero-positive to *Leptospira*. Home slaughter cattle or pigs were significant risk factors for *Leptospira* sero-positivity. There were no clear relationships between the animal species handled at work and sero-status. However, veterinarians spending a "mid to high" proportion of time (>50% to $\leq$75%) with pets had higher odds of being sero-positive than those not working with pets. A borderline positive association (p=0.09) was observed between sero-positivity and clinical influenza-like illness ($\geq$3 days off work) in the 18 months before the study. Awareness of risk factors associated with *Leptospira* sero-positivity may help to reduce *Leptospira* infection in New Zealand veterinarians. Assuming causality, this suggests that 8.3% of these cases may be attributed to *Leptospira* infection.

3.2 Introduction

The importance of leptospirosis as a re-emerging zoonotic disease in some countries has recently been emphasised (Bharti et al. 2003; Adler and De la Peña Moctezuma 2010). The disease is endemic worldwide but a higher incidence is observed in tropical and subtropical climates where the conditions of the temperature and humidity favour the survival of *Leptospira* in the environment. Moreover, most countries in tropical regions are developing countries, where there are usually higher opportunities for contact between potentially infected animals and humans (Pappas et al. 2008; Levett 2004). Leptospirosis is often associated with rodents but all mammal species, including livestock and companion animals, can be infected. Animals can shed the organism in the urine for an extended period. Humans are infected after direct or indirect contact with urine containing the bacteria. Leptospires enter the body through mucosal membranes or abrasions in the skin. Clinical signs of disease vary from mild, self-limiting influenza-like illness lasting 3 to 5 days, to a severe condition that can involve renal and hepatic malfunction, and if not treated can be fatal (Faine et al. 1999). Typical symptoms of the disease include myalgia, fever, nausea, vomiting, and headache (Vickery et al. 2006).

Leptospirosis is endemic in New Zealand livestock. Surveys have estimated seroprevalence in beef cattle, sheep and deer animals ranging from 48%–65%, 33%–48% and 26%–43% for *L. borgpetersenii* serovar Hardjo type Hardjo-bovis (Hardjo) and 25%–35%, 0%–45% and 11%–50% for *L. interrogans* serovar Pomona (Pomona), respectively (Dreyfus et al. 2011; Subharat et al. 2012). Other production animals, including dairy cattle and pigs, are also at risk of leptospirosis but vaccination against serovars Hardjo and Pomona is thought to have largely reduced leptospirosis incidence and the risk of transmission to humans in New Zealand (Marshall 1987; Marshall and Manktelow 2002). However, a recent pilot study in 44 conveniently-selected dairy herds with a long history of vaccination against *Leptospira* (during the last 5 to >20 years), identified 13% urinary shedders by PCR and dark field microscopy in 30% of herds (Wilson et al. 2013). Although serovars were not determined in that study, it has been reported elsewhere that transient urinary shedding of Hardjo can occur in vaccinated cattle (Zuerner et al. 2011). Serovars not included in cattle vaccines and reported from notified human cases in New Zealand include *L. borgpetersenii* serovar Ballum (Ballum) and *L. borgpetersenii* serovar Tarassovi (Tarassovi) (ESR 2002-2014).

Companion animals can also be exposed to *Leptospira*, although their role in transmission of *Leptospira* to humans is unknown. A cross-sectional study in New Zealand dogs found 15.0% of serum submissions sero-positive to Hardjo, Pomona, Ballum, or *L. interrogans* serovar Copenhageni (Copenhageni). The latter serovar was the most prevalent (10.3%). Hardjo (3.5%) ranked second and was predominantly found in farm dogs (Harland et al. 2013). Similar observations were made earlier in healthy dogs from rural or urban environments (O'Keefe et al. 2002).

In New Zealand the incidence of leptospirosis in humans is among the highest in industrialised countries being 2.6 per 100,000 people (Pappas et al. 2008). The most common serovars in notified human cases during 2003–2013 were Hardjo, Pomona and Ballum (ESR 2002-2014). The disease was identified as a common occupationally acquired infectious disease in the country and a higher incidence was described for males compared to women (Thornley et al. 2002). Working in abattoir and livestock farming were the most frequently reported occupations among *Leptospira* cases (ESR 2002-2014). The risk of *Leptospira* exposure for workers of a sheep-only abattoir was quantified in 3–54 carcasses per day. Risk depended on work position and year (Dorjee et al. 2008; Dorjee et al. 2011). The sero-prevalence of Hardjo and Pomona in abattoir workers of another sheep abattoir was estimated in 9.5% (MAT $\geq$24; 2008) (Benschop et al. 2009). During 2009/10 a more comprehensive sero-survey of abattoir workers estimated that 10.9% of the workers were sero-positive to Hardjo or Pomona (MAT $\geq$48): 5.4% in beef, 12.9% in sheep and 17.5% in deer abattoir workers (Dreyfus et al. 2014).

Veterinarians are not commonly listed among notified cases. During 2003–2013, only two veterinary-related cases (1 veterinary technician in 2012 and a veterinarian in 2013) were notified (ESR 2002-2014). However, veterinarians are likely at high risk of leptospirosis due to the potential exposure to urine of infected animals. A previous study conducted in New Zealand found 1/86 veterinarians sero-positive to Hardjo (MAT $\geq$100) (Robinson and Metcalfe 1976). Another sero-survey found no antibody titres for *Leptospira* in 302 veterinary students during 2010/11 at the MAT titre of *geq*48 (Fang et al. 2014) but no recent estimate of the extent of sero-positivity to *Leptospira* in veterinarians was available. This study aimed to estimate the sero-prevalence of five *Leptospira* serovars and to evaluate risk factors for sero-positivity in New Zealand veterinarians.

3.3 Materials and Methods

3.3.1 Study Design

A cross-sectional study was designed to estimate sero-prevalence of *Leptospira* and assess risk factors associated with *Leptospira* sero-status. Veterinarians attending the Deer special Interest group conference of the New Zealand Veterinary Association in Queenstown (May 2012) or the New Zealand Veterinary Association annual conference in Hamilton (June 2012) were eligible for inclusion. A station was set up in the exhibition hall of each conference where certified phlebotomists sampled blood from voluntary participants. Samples were kept refrigerated during the day at 4°C. Tubes with blood were sent overnight inside bio-bottles™ to the Hopkirk Institute, Massey University for serum extraction. Sera were kept frozen until testing. A modified microscopic agglutination test (MAT) as described by Faine et al. (1999) was used to detect antibodies against Hardjo, Pomona, Ballum, Copenhageni and Tarassovi. In summary, eight 2-fold dilutions of serum were made using sterile 0.9% saline solution as a diluent. An equal volume of live *Leptospira* antigen was then added to each dilution to make a final serial dilution that ranged from 1:24 to 1:3072. The last dilution able to agglutinate more than 50% of leptospires was taken as the final titre for each serovar.

3.3.2 Recording of risk factors

A questionnaire (Appendix V: Veterinarian questionnaire) was designed to capture information about exposure to potential risk factors during the previous 18 months. This time-frame of exposure was selected based on the duration of titres previously estimated as 10-months for Pomona and 29-months for Hardjo (Dreyfus et al. 2015). The questionnaire was available for participants at the conferences either online (Survey Gizmo®) or paper. It covered personal information, animal exposure in the work place, animal and environmental exposure outside the work place, previous leptospirosis illness, influenza-like illness in the last 18 months, and general opinions about leptospirosis.

Influenza-like illness was defined as an episode of illness accompanied by some or all of the following signs/symptoms: fever, headache, myalgia, photophobia, sweating and severe general debility.

3.3.3 Statistical analysis

Antibody titres (24 to 3072) were described graphically using numbers (Figure 3.1). Sero-prevalence and 95% confidence intervals were estimated using the titre cut-off of $\geq$48 for a positive sample.

Gender and age were described using percentages and summary statistics (median, minimum value (min), 1st quartile (1st Q), 3rd quartile (3rd Q) and maximum value (max)), respectively. Differences in the time spent working with different animal species between females and males were assessed using the Wilcoxon rank-sum test. The association between gender and home slaughter of pigs or cattle was explored using logistic regression.

The percentage of time spent in different activities within the veterinary practice was described and kernel density plots were used to graphically compare percentages in sero-positive and sero-negative veterinarians.

The number of previous leptospirosis episodes and the most common signs and symptoms associated with these episodes were recorded. The association between *Leptospira* sero-status and past episodes of leptospirosis was explored using prevalence ratios.

The number of influenza-like illnesses in the last 18 months was recorded. Influenza-like illness was categorised according to the time veterinarians spent off work due to this illness in categories: no illness; off work less than three days; and off work three days or more. The relationship between *Leptospira* sero-status and incidence of influenza-like illness (yes/no) or time off work categories (None, $<$3 days, $\geq$3 days) was explored. The population attributable fraction (PAF) was estimated. PAF is a measure of effect in the population. In this case it estimates the percentage of influenza-like illness (PAF) in the population during the last 18 months that may be attributed to *Leptospira* infection.

For inclusion in the multivariable analyses, the percentage of time spent in contact with different animal species was categorised as: none (0%), low ($>$0% to 25%),

mid-low (>25% to 50%), mid-high (>50% to 75%), and high (>75%). For example, a veterinarian could be exposed to dairy cattle for 60% (mid-high) of his/her working time, to pets for 10% (low) and office work for the remaining 30% (mid-low) of work related time.

Unconditional associations between each of the putative risk factors and *Leptospira* sero-status were evaluated using logistic regression. Variables with a p-value $\leq$0.35, tested by the likelihood ratio test (LRT), were selected for inclusion in the multivariable logistic model. Backward elimination of variables from a full multivariable model was based on the LRT with significance threshold of p>0.05. Akaike's information criterion was used to select the final multivariable model (Akaike 1974). Goodness of fit of the multivariable model was assessed using Pearson and deviance residuals χ^2 and Le Cessie-van Houwelingen test (Cessie and Houwelingen 1991). Observations with large Pearson residuals and delta-beta values were removed temporarily to investigate their influence on the coefficients and standard errors of the final multivariable model.

Analyses were performed using R software version 3.03 (R Core Team 2014).

3.3.4 Human ethics application

Ethical approval was obtained from the Massey University Human Ethics Committee (MUHEC: Southern A Application - 12/09).

3.4 Results

Data from 277 veterinarians, working in New Zealand that were sampled and had completed the questionnaire, were used for analysis.

3.4.1 Titre distribution

Antibody titres for *Leptospira* (MAT ≥48) were detected in 14 veterinarians. The most frequent serovar was Pomona (n=7) followed by Hardjo (n=6). Antibodies detected for Pomona had a maximum titre of 96, while for Copenhageni and Ballum the maximum titre was 48. Comparatively higher antibody titres of up to 1536 were observed for Hardjo. Figure 3.1 shows the titre distribution for Hardjo, Pomona, Ballum, Copenhageni and Tarassovi.

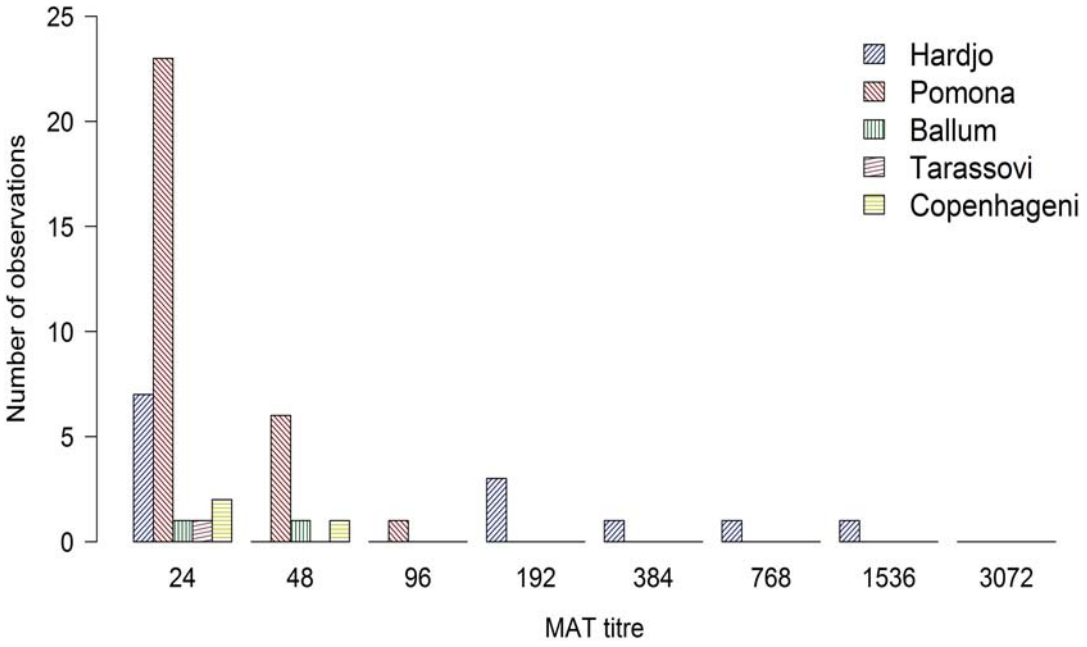

Figure 3.1: Distribution of the number of samples at each microscopic agglutination test titre for Hardjo, Pomona, Ballum, Copenhageni and Tarassovi.

3.4.2 Sero-prevalence

Sero-prevalence of *Leptospira* (≥48 MAT titre), all serovars included, was 5.1% (95% CI 2.8%–8.3%). One participant was sero-positive to both Hardjo and Pomona.

Table 3.1: Number of participants sero-positive and negative and sero-prevalence (% and 95% CI) for individual serovars at a titre of ≥48.

Serovar	n positive	n negative	Sero-prevalence (95% CI)
Pomona	7	270	2.5 (1.0–5.1)
Hardjo	6	271	2.2 (0.8–4.7)
Ballum	1	276	0.4 (0.0–2.0)
Copenhageni	1	276	0.4 (0.0–2.0)
Tarassovi	0	277	0.0 (0.0–2.0)
Overall	14	263	5.1 (2.8–8.3)

[a] One veterinarian sero-positive to Hardjo and Pomona.

No veterinarian was sero-positive to Tarassovi. Table 3.1 summarises sero-prevalence for each serovar individually and for any of the 5 serovars.

3.4.3 Age and gender

The median age of study participants was 42 years (min=22 years; 1st Q=33, 3rd Q=53, max=73 years).

Thirty-nine per cent of veterinarians were females (n=109). Male participants were in general older (median 47 years, min=23, 1st Q=37, 3rd Q=57, max=73) than female participants (median=35 years, min=22, 1st Q=29, 3rd Q=45, max=59). There was no statistically significant difference (p=0.40) between the sero-prevalence in men (10/168 positives) and women (4/109 positives). Females spent significantly (p¡0.001) more time on average working with dogs and cats (23.5%; 95% CI 17.5%–29.6%) than males (11.4%; 95% CI 8.2%–14.6%). On the other hand, males spent significantly (p<0.001) more time working with deer (6.7%; 95% CI 4.1%–9.3%) than females (2.7%; 0.5%–5.0%). Time spent with dairy cattle, beef cattle, and sheep was not significantly different between males and females. Home slaughter of cattle (p=0.54) or pigs (p=0.41) were not associated significantly with gender.

3.4.4 Occupational exposure to animals

Most veterinarians sampled (n=155) worked at least a quarter of their time with dairy cattle. In contrast, a small proportion of veterinarians worked more than 25% of their time with beef (n=18), sheep (n=15), or deer (n=12). Few (n=24) worked 100% of their time with a single species, while 22 had no animal contact at work. Figure 3.2 shows the smoothed distribution of the proportion of time that sero-

positive and sero-negative veterinarians spent working with different animal species. These plots suggest that sero-positive veterinarians were not more involved than sero-negative veterinarians in any of the extreme-ends of exposure to any species, but some differences were apparent at middle portions of exposure to some species.

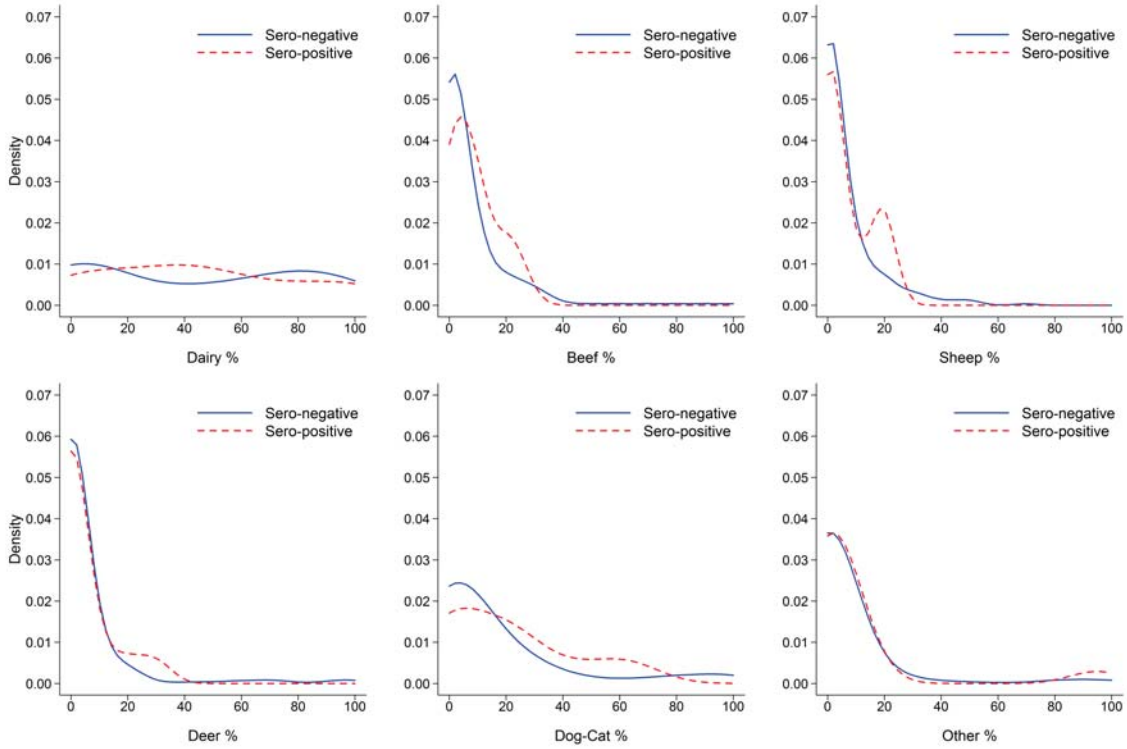

Figure 3.2: Smoothed proportion of sero-positive and sero-negative participants (density) by percentage of time spent in contact with different animal species at work.

3.4.5 History of previous leptospirosis episodes

Leptospirosis was previously diagnosed by general practitioners and laboratory test (serology) 11 times in 10 participants. Nine were able to recall symptoms of disease, which were: headache (n=9), sweating (n=9), fever (n=8), myalgia (n=7), sore eyes (n=6), photophobia (n=6), severe debility (n=5) and meningitis (n=1). Eight recalled being off work a median time of 7 days (min=2 days, 1st Q=3 days, 3rd Q=21 days, max=105 days) and one recalled being hospitalised because of the disease. Eight of these episodes of leptospirosis occurred from 43 years to 16 years prior to participation in this study, while one occurred three months before sampling. Having clinical leptospirosis in the past was significantly associated ($p<0.001$) with

Table 3.2: Association between *Leptospira* sero-status and time off work due to influenza-like illness in the last 18 months.

Sero-status	**≥3 days ill**	**<3 days ill**	**Not ill**	**PR[a](95% CI)**	**PR[a](95% CI)**
Positive	3	5	6	1.4 (0.8–2.2)	2.8 (0.9–8.2)
Negative	20[c]	86[c]	151	**PAF[b](95% CI)**	**PAF[b](95% CI)**
Total	23	91	157	1.8 (⁻1.6–5.0)	8.3 (⁻6.3–20.9)

[a]Prevalence ratio (PR), and population attributable fraction (PAF) were estimated using values for influenza-like illness and not ill in the last 18 months. For PR and PAF, 95% confidence intervals are reported.
[b]Prevalence ratio (PR), and population attributable fraction (PAF) were estimated using values for ≥3 days ill and <3days or not ill in the last 18 months. For PR and PAF, 95% confidence intervals are reported.
[c]Four veterinarians, sero-negative to *Leptospira*, had influenza-like illness but did not state number of days off work.

sero-positivity (prevalence ratio (PR)=10.7; 95% CI 4.0–28.3). This strong association was also observed when excluding the recent episode of leptospirosis (PR=8.9; 95% CI 2.9–26.9). Previous leptospirosis was excluded from the multivariable model since it could mask the effect of other risk factors associated with sero-status.

3.4.6 Influenza-like illness in the last 18 months

A total of 118/275 veterinarians experienced influenza-like illness in the previous 18 months. Among *Leptospira* sero-positives, 8/14 had influenza-like illness in the last 18 months compared with 110/261 in sero-negative veterinarians. Influenza-like illness cases in sero-positive veterinarians was therefore 1.4 times greater than in sero-negative veterinarians (p=0.28). However, sero-positive veterinarians were more likely to spend three days or more off work due to influenza-like illness than sero-negative veterinarians (Table 3.2) (p=0.09). Assuming a causal relationship between *Leptospira* sero-positivity and influenza-like illness, the percentage of influenza-like illness in the last 18 months associated with ≥3 days off work that was attributable to *Leptospira* infection in the population of veterinarians, was 8.3% (PAF). The influenza-like illness variable was excluded from the multivariable model as it was likely to be a consequence of leptospirosis rather than a cause, hence not a confounder for other risk factors.

3.4.7 Unadjusted associations

Unadjusted ORs (Table 3.3) suggested that home slaughtering of cattle, sheep or deer in the previous 18 months significantly increased the odds of sero-positivity compared with veterinarians who were not involved in such activities. Categories of

Table 3.3: Unadjusted associations between potential risk factors and *Leptospira* sero-status (likelihood ratio test p-value (p-LRT)≤0.35).

Variable	**Levels**	**n**	**OR (95% CI)[a]**	**p-LRT**
Dairy exposure	High	77	2.6 (0.3–30.0)	0.3
	Mid-High	40	3.4 (0.3–39.0)	
	Mid-Low	38	7.7 (0.8–71.1)	
	Low	56	5.0 (0.5–46.1)	
	None	66	Reference	
Dog-Cat exposure	High	19	NA[b]	0.22
	Mid-High	9	6.3 (1.0–38.7)	
	Mid-Low	24	2.0 (0.4–11.1)	
	Low	109	1.1 (0.3–3.8)	
	None	116	Reference	
Home slaughter cattle	Yes	32	4.8 (1.5–15.2)	0.02
	No	240	Reference	
Home slaughter pigs	Yes	11	8.5 (2.0–36.6)	0.01
	No	261	Reference	
Home slaughter sheep	Yes	65	3.5 (1.2–10.2)	0.03
	No	207	Reference	
Hunting wild deer	Yes	55	2.4 (0.8–7.3)	0.16
	No	221	Reference	
Camping lakes/rivers	Yes	120	2.5 (0.8–7.6)	0.11
	No	157	Reference	
Fishing	Yes	59	3.0 (1.0–8.9)	0.06
	No	218	Reference	
Own deer	Yes	15	3.2 (0.7–15.8)	0.20
	No	262	Reference	
Own pigs	Yes	16	3.0 (0.6–14.6)	0.23
	No	261	Reference	

[a] Odds ratio and 95% confidence intervals.
[b] not identifiable as no sero-positives were observed.

the time spent with beef cattle, sheep, deer or other animals; hunting wild pigs, wild goats, rabbits, or birds; owning cattle, sheep, horses, dogs or cats; home slaughter of deer; age and gender were largely not associated (p-LRT>0.35) with *Leptospira* sero-status (results not shown in Table 3.3).

3.4.8 Multivariable analysis

In the final multivariable model, home slaughter of cattle or pigs was positively associated with *Leptospira* sero-positivity. For example, the odds of being sero-positive for veterinarians involved in home slaughter of cattle in the previous 18 months were 4.6 times the odds of being sero-positive for those not involved in this

Table 3.4: Results of a multivariable logistic regression model between *Leptospira* sero-status and potential risk factors.

Risk factor	**Levels**	**OR (95% CI)[a]**	**p-LRT[b]**
Dog-Cat exposure	High	NA[c]	0.17
	Mid-High	9.2 (1.4–62.8)	
	Mid-Low	2.5 (0.4–14.9)	
	Low	1.1 (0.3–4.3)	
	None	Reference	
Home slaughter cattle	Yes	4.6 (1.3–16.1)	0.03
	No	Reference	
Home slaughter pigs	Yes	7.9 (1.7–37.5)	0.02
	No	Reference	

[a] Odds ratio and 95% confidence intervals.
[b] likelihood ratio test p-value.
[c] not identifiable as no sero-positives were observed.

activity, after controlling for the effects of home slaughter of pigs and occupational exposure to dogs and cats. Also, veterinarians spending from 50% to 75% of their time working with dogs or cats had significantly higher odds of being sero-positive than those not working with dogs or cats (Table 3.4).

3.5 Discussion

In this sample of veterinarians, 14/277 were sero-positive (5.1%; 95% CI 2.8%–8.3%). A previous study investigated the sero-prevalence in New Zealand veterinarians for Hardjo, Pomona, Ballum, Copenhageni, Tarassovi, plus serovars Canicola, Autumnalis, Andaman and Medanensis not tested in our study, observing 1/86 (1.2%) veterinarians sero-positive to Hardjo (Robinson and Metcalfe 1976). The MAT titre cut-off used in that investigation was one dilution higher (≥100) than the one used in the present study. At an equivalent MAT titre of 96, 7/277 veterinarians were sero-positive (2.5%; 95% CI 1.0%–5.1%); which is similar to that of the previous study.

The selection of the MAT titre cut-off depends on the purpose for testing. For a clinical case of leptospirosis based on MAT, the World Health Organisation (WHO) considered a MAT titre of ≥400 in a single or paired serum sample, or a 4-fold increase in MAT titre in acute and convalescent phase in subjects experiencing clinical signs compatible with leptospirosis (WHO 2011). There is not a defined cut-off for antibody MAT titres when assessing *Leptospira* sero-positivity in apparently healthy individuals working in high risk occupations, hence the cut point must be considered when comparing sero-prevalence studies. For example, sero-prevalence to Hardjo and Pomona in apparently healthy abattoir workers was estimated in 10.9% (95% CI 8.5%–13.9%) using the MAT titre of ≥48 (Dreyfus et al. 2014), which is 2.5 times higher than the sero-prevalence observed to these two serovars in veterinarians (4.3%, 95% CI 2.3%–7.5%). Sero-prevalence to Hardjo, Pomona and Ballum has also been assessed in apparently healthy veterinary students where none of the 302 participants was sero-positive to these serovars at a titre of ≥48 (Fang et al. 2014). This finding suggests that exposure to *Leptospira* most likely occurs during the years of active work.

Leptospirosis is likely under-ascertained in New Zealand, especially for mild infections. The disease in people is characterised by non-specific symptoms (Levett 2004) that may be confused with a influenza. We observed a marginally significant association between *Leptospira* sero-positivity and influenza-like illness (≥3 days off-work) in the previous 18 months. Although, the cross-sectional design used does not allow assessment of when exposure and outcome occurred; we believe that some of the influenza-like illnesses recorded are a consequence of *Leptospira* infection. This

is consistent with recent findings in abattoir workers where new Hardjo or Pomona infections increased the risk of influenza-like illness by 1.9 (95% CI 1.3–2.7) in a year and the proportion of influenza-like illness cases attributed to Hardjo or Pomona infection (PAF) was 10.0% (Dreyfus et al. 2015). If we assume a causal relationship between *Leptospira* sero-positivity and being off work for ≥3 days with influenza-like signs of illness in the last 18 months, it can be said that 8.3% of these cases could be prevented if *Leptospira* exposure of veterinarians was controlled (Table 3.2).

A significant risk factor for *Leptospira* sero-positivity was home slaughtering cattle (n=32) or pigs (n=11). The average predicted sero-prevalence increased from 3.2% (95% CI 1.0%–16.9%) in veterinarians not performing these activities to 16.4% (95% CI 4.4%–46.0%) in veterinarians performing any of these activities. This result contrasts with findings from the sero-survey in veterinary students of New Zealand, where no student was sero-positive and yet 20.9% stated that they performed home slaughter in the 18 months prior to sampling (Fang et al. 2014). A plausible explanation for the difference observed between students and veterinarians is the presumably shorter length of time that students were exposed to home slaughter due to their young age. A recent study of abattoir workers did not find a significant association between home slaughter and *Leptospira* sero-positivity (Dreyfus et al. 2014) but no information was available about the species slaughtered at home or the frequency of this practice. Possibly, the additional risk of *Leptospira* exposure from home slaughter in abattoir workers was relatively small compared with veterinarians.

Home slaughter of sheep was not a significant risk factor in the multivariable model, most likely due to its significant association with slaughtering cattle (OR=2.5, p=0.02) and pigs (OR=9.5, p=0.001). Hence, those involved in home slaughter appear to process more than one species. It is therefore impossible to make inferences about which home slaughtered species was the likely source of infection for veterinarians.

A positive correlation between *Leptospira* sero-prevalence and the time spent with a specific species was expected, especially since earlier studies demonstrated *Leptospira* sero-positivity in livestock (Dreyfus et al. 2011; Subharat et al. 2012) and that between 21% and 41% of sero-positive animals potentially shed *Leptospira* in urine (Dorjee et al. 2008; Fang et al. 2015). Instead, sero-prevalence tended to be higher when veterinarians worked with multiple species (Figure 3.2). If any species,

it was the contact with pets and dairy cattle that tended to be positively associated with sero-positivity. However, the data are too sparse to draw clear conclusions about the comparative risk of infection from any particular animal species that veterinarians are handling during their clinical work.

In New Zealand dogs, Copenhageni was the most commonly observed serovar, followed by Hardjo which was commonly present in dogs from rural locations or farm working dogs (Harland et al. 2013; O'Keefe et al. 2002). However, only one veterinarian (who did not work with dogs or cats) was sero-positive for Copenhageni. This contrasts with six veterinarians sero-positive to Hardjo; three of whom had no or minor exposure to dogs or cats (0%–3%), and the other three had moderate exposures (20%–65%). Furthermore, none of the 19 veterinarians who worked >75% of the time with dogs or cats were sero-positive.

A strong and highly significant positive association between previous leptospirosis episodes and sero-positivity to *Leptospira* observed in this survey was also reported from abattoir workers (Dreyfus et al. 2014). Antibodies are usually detectable from 5 to 10 days after infection but they may persist for months or even years after infection at low antibody titres (Cumberland et al. 2001). Re-testing of 69 meat inspectors with previous positive titres, irrespective of their clinical history, showed that MAT titres of 384–192, 96, and 48–24 persisted for at least 30, 43 and 52 months, respectively in some individuals (Blackmore et al. 1984). Nevertheless, the persistence of titres is difficult to assess because often individuals at high risk of infection are studied and it is unknown whether persistent titres are the product of a slow decay, or continued re-exposure. The latter hypothesis could be questionable since sero-conversion in sero-negative meat inspectors was estimated in 4.4% a year (Blackmore et al. 1984). Previous episodes of clinical leptospirosis in veterinarians of this study occurred between 16 and 43 years before sampling; it is therefore unlikely that the strong association between previous leptospirosis and sero-positivity was due to a slow decay of antibodies, thus they may rather reflect continuous re-exposure to *Leptospira* resulting in mild or subclinical infections.

Gender was not significantly associated with *Leptospira* sero-positivity (p=0.4) in this group of veterinarians. National data show males have a higher incidence of leptospirosis than females which may be due to the predominance of males in high risk occupations (Thornley et al. 2002). For example, male abattoir workers

were more likely to be sero-positive and sero-converted more often to *Leptospira* than female workers (Dreyfus et al. 2014; Dreyfus et al. 2015). In our data, females represented 39.4% of participants and they spent on average more time with dogs or cats and less time with deer than males. Other occupational exposures and the proportion of veterinarians involved in home slaughter cattle or pigs were not different between females and males.

The 277 participant veterinarians represented 11% of the veterinarians registered in New Zealand (n=2521) in the year of the study (Anonymous 2012). The median age of veterinarians in New Zealand was 43 years, similar to our study (42) but a slightly higher proportion of females (62%) and males (31%) under 40 years was observed in comparison to population demographics of veterinarians (59% and 23%, respectively). Potential selection bias may have been introduced by design as only veterinarians attending the conferences had the opportunity to participate in the study. The effect this might have had on sero-prevalence and ORs is difficult to predict and subject to speculation. Nonetheless, we can see no reason to believe that selection bias affected the overall conclusions presented in this study.

3.6 Conclusion

Leptospira sero-prevalence in veterinarians is about half as high as that of abattoir workers. High risk activities for *Leptospira* sero-positivity in veterinarians included home slaughter of cattle or pigs. No clear associations were found with the animal species that veterinarians were in contact with during professional activities. The borderline association between *Leptospira* sero-positivity and influenza-like illness resulting in $\geq$3 days off work suggested that 8.3% of these influenza-like cases were attributable to *Leptospira* infection during the 18 months prior to sampling. However, caution must be taken when interpreting this result since the cross-sectional design does not warrant a causal relationship.

3.7 Acknowledgement

We express our gratitude to all veterinarians that voluntarily participated in the study and made it possible, to Neville Haack for all the technical assistance and support provided, and to MSD Animal Health, Zoetis and Virbac for computers and online connection for participants to access the questionnaire.

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Chapter 4

Estimating the burden and economic cost of leptospirosis in New Zealand

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4.1 Summary

Leptospirosis in humans is notifiable in New Zealand and mainly affects people in close contact with domestic livestock. The disease has an impact on both human health and animal production that has not been quantified at the population level. Stochastic simulation was used to estimate the burden of leptospirosis in terms of disability-adjusted life years (DALYs), and the economic cost associated with monetary loss due to absence from work, treatment of disease, animal production loss, and cost of vaccination. A longitudinal study of abattoir workers and two cross-sectional studies of farmers and veterinarians, reporting annual risks of influenza-like illness attributable to *Leptospira* infection were used to estimate the number of leptospirosis cases expected in a year. The number of days absent from work and treatment cost were informed by results of previous studies and Accident Compensation Corporation (ACC) data. The cost of lost animal production was based on results of observational studies in beef cattle, sheep and deer conducted in New Zealand. The median annual number of expected severe and mild human cases of leptospirosis was 1,567 (95% probability interval (95% PI) 919–2,542). Median annual DALYs were 0.43 (95% PI 0.05–3.12) per 100,000 people for the entire population, and 16.76 (95% PI 2.06–122.2) per 100,000 people working in at risk occupations (i.e. abattoir workers, farmers and veterinarians). Human infection resulted in a median cost of 4.49 (95% PI 1.55–11.49) million New Zealand dollars (NZ$) due to absence from work and disease treatment. Median production loss cost in beef cattle, sheep, and deer was NZ$11.31 (95% PI 5.50–22.29) million, while median vaccination cost in cattle, (including dairy), sheep, and deer was NZ$8.99 (95% PI 8.19–10.00) million. The total annual cost of leptospirosis was NZ$25.36 (95% PI 17.99–37.65) million, equivalent to NZ$597,910 (95% PI 424,060–887,500) per 100,000 people. This study provides an estimate of the cost and burden of leptospirosis in New Zealand that informs public health authorities and livestock industries for the appraisal of interventions and preventive measures, and provides a model for zoonotic disease burden estimation that could be adapted to resource-poor settings.

4.2 Introduction

Leptospirosis is a global zoonosis that affects at least about 15 per 100,000 people annually worldwide (Costa et al. 2015). The incidence of leptospirosis in New Zealand is one of the highest among the so called "developed" countries (Pappas et al. 2008). From 2001 to 2014, an annual average of 91 cases was notified, equivalent to 2.2 cases per 100,000 people in New Zealand (ESR 2002-2015). Whereas human leptospirosis rate is typically higher in tropical regions and under low socio-economic conditions (Costa et al. 2015), in New Zealand it affects predominantly people occupationally exposed to domestic livestock species. Between 2001 and 2014, 81.9% of notified cases were either farmers or abattoir workers (ESR 2002-2015).

In humans, leptospirosis can cause severe illness that may result in renal failure, pulmonary haemorrhagic syndrome, and ultimately death (Gouveia et al. 2008; Yersin et al. 2000; Levett 2001). However, most infections result in sub-clinical or mild disease resembling influenza, dengue or other febrile illnesses (Bharti et al. 2003; Levett 2001). Duration of acute illness usually last from one to three weeks (Goris et al. 2013; McClain et al. 1984; Dreyfus et al. 2014). Leptospirosis can also result in chronic sequelae that could last for a variable period of time. In The Netherlands, Goris et al. (2013) observed that 30.2% of leptospirosis cases suffered from persistent sequelae that lasted for $\leq$2 months in 19.3% of the cases and >24 months in 21.1% of the cases. Although duration of illness and time off-work resulting from mild leptospirosis are difficult to quantify, recent serological studies in abattoir workers, veterinarians and farmers have positively associated *Leptospira* sero-status and influenza-like illness (Sanhueza et al. (2015), Dreyfus et al. (2015b), and Chapter 2). From those studies, estimates of the annual risk of illness due to *Leptospira* infection (population attributable risk; PAR) were the basis for estimating the expected number of annual cases in these at risk populations.

The burden of a disease can be quantified in terms of disability-adjusted life years (DALYs), and the economic cost of disease due to days absent from work, hospitalisation and treatment in monetary terms. DALYs is a health measure used to quantify the burden of disease, representing the number of healthy life years lost due to illness and can be directly compared with other DALYs estimates to prioritise interventions and preventive measures. For leptospirosis, the global burden was estimated at 41.8 DALYs per 100,000 people a year (Torgerson et al. 2015).

However, no estimate is currently available for the burden and cost of leptospirosis in New Zealand.

Leptospirosis in cattle has been associated with a variety of production losses in the past, although not without controversy. For example, Hoare and Claxton (1972) observed mastitis and decreased milk yield in a leptospirosis outbreak of serovar Hardjo, whereas another investigation did not show an effect on milk yield for infections with this serovar (Dhaliwal et al. 1996). Guitian et al. (1999) observed an increased time from calving to conception in sero-positive cattle to serovar Hardjo compared to sero-negative herd mates, although the authors did not observe an association with abortions, in contrast to other investigations that related *Leptospira* sero-positivity (Hardjo and Pomona) to foetal loss (Elder et al. 1985; Johnson et al. 1974). Differences in infecting serovars and the endemic scale of infection may explain these contrasting reports. In New Zealand, sero-positivity to *Leptospira borgpetersenii* serovar Hardjo (Hardjo-bovis) and *Leptospira interrogans* serovar Pomona (Pomona) is highly prevalent in beef cattle, sheep, and deer (Dreyfus et al. 2011; Ayanegui-Alcerreca et al. 2010). Additionally, foetal loss was associated with Hardjo-bovis and Pomona sero-positivity in beef cattle (Sanhueza et al. 2013), and Pomona sero-positivity in one sheep farm (Ridler et al. 2015). Furthermore, vaccination trials in deer suggested an economic benefit of immunisation against leptospirosis due to increased growth rate and weaning percentage (Subharat et al. 2011; Subharat et al. 2012). To a lesser extent, reduced growth has also been observed in unvaccinated beef cattle and sheep compared with vaccinated animals in some New Zealand farms (Vallée et al. 2014).

In this study, we estimated the burden of leptospirosis in New Zealand in terms of DALYs, and economic cost due to lost work days, hospitalisation and treatment of human illness, animal production loss, and vaccination cost against leptospirosis in cattle, sheep, and deer.

4.3 Materials and Methods

4.3.1 Simulation model

Based on the parameters and bibliographic sources described below, a stochastic simulation model was developed in R (R Core Team 2015) version 3.2.1 to estimate the cost and burden of leptospirosis in New Zealand (Appendix VI: Burden and cost Code). Assumptions are listed in Appendix VII: Burden and cost assumptions. One hundred-thousand random samples were taken from each of the distributions used in the simulation model. Results are presented using medians and 95% probability intervals (95% PI).

4.3.2 Burden of leptospirosis

The burden of leptospirosis was estimated in terms of DALYs.

Disability-Adjusted Life Years (DALYs)

DALYs is a measure of years of healthy life lost due to disease estimated by adding years of life lost (YLL) and years lost due to disability (YLD).

$$DALYs = YLL + YLD \tag{4.1}$$

The first argument in Formula 4.1 (YLL) takes into account mortality due to leptospirosis, which is extremely low in New Zealand and therefore ignored. The second argument in DALYs estimation (Formula 4.1) is YLD. It quantifies to what extent a person is disabled during illness and it is calculated using Formula 4.2, where I represents the number of incident cases; DW the disability weight, indicating how ill or disable a person is during illness; and D the duration of illness.

$$YLD = I \times DW \times D \tag{4.2}$$

Annual incidence of leptospirosis

Estimates of the association between *Leptospira* sero-status and influenza-like illness were obtained from three studies targeting occupational groups at risk of

leptospirosis in New Zealand. The first investigation focused on abattoir workers that were blood sampled twice with an adjusted interval of one year to assess sero-conversion to *Leptospira* serovars Hardjo-bovis and Pomona and the associated incidence of influenza-like illness (Dreyfus et al. 2015b). The other two investigations addressed the sero-prevalence of Hardjo-bovis, Pomona, *Leptospira bogpetersenii* serovars Ballum (Ballum) and Tarassovi (Tarassovi), and *Leptospira interrogans* serovar Copenhageni (Copenhageni) among veterinarians (Sanhueza et al. 2015) and farmers of beef cattle, sheep, and deer (Chapter 2). In both studies, participants were asked to recall influenza-like illness episodes in the 18 months prior to sampling.

The population attributable risk (PAR) is a measure of association that indicates the incidence risk of disease in the population over a specified time period that can be attributed to exposure. It is calculated as the incidence in the total population minus the incidence in the non-exposed group. PAR was approximated from these three studies as the risk of influenza-like illness associated with *Leptospira* seropositivity. A Bayesian model was used to estimate PAR and its 95% probability intervals (Pirikahu et al. 2016). The estimated PARs of farmers and veterinarians were adjusted to a one-year incidence by a factor of 2/3 since the participants had been questioned about influenza-like illness for an 18 months period.

The population of abattoir workers (n=16,224), people farming dairy or beef cattle, sheep, and/or deer (n=70,461) and veterinarians (n=1,989) in New Zealand were obtained from the 2013 census according to the Australian and New Zealand Standard Classification of Occupations (ANZSCO) (Anonymous 2013). The number of farmers at risk of leptospirosis was reduced using estimates of vaccine coverage in each livestock species (see Cost of vaccination for details) and mean vaccine efficacy of 82% (Chapter 5). The number of farmers was multiplied by one minus the proportion of farms that vaccinate against leptospirosis and the PAR to obtain the expected number of farmers having influenza-like illness attributed to *Leptospira* infection each year. Prevalence data were not available for dairy cattle workers. Thus, to estimate leptospirosis incidence, the specific PAR for farmers of beef cattle, sheep and/or deer was applied to this occupational group.

The PAR for groups not at risk of occupational leptospirosis, i.e. without occupational contact with animals, could not be approximated from available data as

equivalent studies had not been done for such groups, so public surveillance data were used. During the last 14 years, on average 18.1% of notified cases were people in low occupational risk groups (ESR 2002-2015). The number of leptospirosis cases in abattoir workers, farmers and veterinarians was assumed to reflect 81.9% of the cases in the population. Hence, the number of cases in occupational groups not at risk of leptospirosis was calculated as the expected number of cases in high risk occupational groups multiplied by the ratio 18.1/81.9. The total New Zealand working population used in the simulation was 3,670,750 people (Anonymous 2016).

Based on data of Dreyfus et al. (2015b), a proportion of 0.136 (95% CI 0.048–0.333) of incident cases was assumed to develop a severe illness and the remaining 0.864 a mild influenza-like illness. Uncertainty was added by fitting a Beta distribution with mode of 0.136 and upper 95% CI as 95% upper boundary. The number of mild infections was the difference between the total number of cases and the number of severe cases. It was also assumed that 30.2% (95% CI 24.2–36.2) of the severe cases would experience post-acute sequelae of disease (Goris et al. 2013). For this, a Beta distribution of mode 0.302 and 95% upper boundary of 0.362 was used.

Duration of illness and disability weights

An acute episode of severe illness was observed to last for 16 days (Goris et al. 2013). To simulate this value, a Poisson distribution with a mean of 16 days was used. For the duration of mild illness, a Poisson distribution with a mean of four days was used. This value was approximated from the 4.4 days off-work that abattoir workers spent due to influenza-like illness (Dreyfus et al. 2015b).

The duration of persistent complaints, observed in The Netherlands by Goris et al. (2013), was used to simulate the duration of post-acute sequelae. For this, a Geometric distribution with a probability parameter of 0.065 was used in the stochastic simulation.

The 95% confidence intervals for disability weights for leptospirosis were taken from values observed for infectious diseases after an acute severe illness (0.139–0.298), acute moderate illness (0.033–0.081) and post-acute sequelae (0.170–0.355) (Salomon et al. 2012). Uniform distributions were fitted to simulate these values

using lower and upper boundaries as minimum and maximum values.

4.3.3 Cost of leptospirosis

Direct and indirect costs of leptospirosis in humans were incorporated in the simulation using hospitalisation and treatment cost and the estimated cost of workplace absence due to illness, respectively. Indirect costs of leptospirosis due to loss of production in beef cattle, sheep, and deer were also estimated. The current vaccination cost to reduce the risk of leptospirosis in these farming systems plus dairy cattle was estimated and added to the total cost of disease.

Cost of human leptospirosis

The cost of human leptospirosis was estimated using two components: the average cost of a work day lost due to illness and the cost of leptospirosis treatment and hospitalisation.

According to the New Zealand income survey (Anonymous 2015b), the median hourly earnings for the June 2015 quarter was 22.83 New Zealand dollars (NZ$) or approximately NZ$183 for eight hours work a day. This value was multiplied by the number of days ill for severe and mild cases, and the number of incident cases for severe and mild episodes. It was also assumed that people experiencing post-acute sequelae were unable to return to work during the first two months only.

Treatment costs of leptospirosis were sought from the Accident Compensation Corporation (ACC) of New Zealand. ACC indicated a minimum average of NZ$3,400 and a maximum average of NZ$23,000 per accepted leptospirosis claim. A uniform distribution using these amounts as minimum and maximum values was used to simulate these data. It was assumed that one third of the severe cases would incur this amount, another third would cost half of this value, and the last third of severe cases would cost a quarter of this value. Finally, it was assumed that half of the mild cases would cost 1% of the ACC treatment cost (NZ$120).

Production loss cost in livestock

Beef cattle Vallée et al. (2014) observed a borderline significant (p=0.07) reduced live weight of 14kg in unvaccinated 19-month-old heifers compared with vaccinated cattle on 1/7 (14%) farms studied. A normal distribution of mean 14kg and an assumed standard deviation of 5kg were used to simulate reduced weight at slaughter due to leptospirosis. A Beta distribution of mode 0.14 and 95% upper boundary of 0.51 was used to simulate the percentage of sero-positive farms experiencing this type of loss in the population (1/7 farms). Dressing percentage was simulated using a Beta distribution with a mode of 0.45 and a 95% upper boundary of 0.53 (Nicola Schreurs, personal communication, 2016). The value per kilogram of carcass weight was simulated using a normal distribution with a mean of NZ$4.5/kg and a standard deviation of NZ$1.5/kg (Nicola Schreurs, personal communication, 2016; `www.interest.co.nz/rural`).

The risk of foetal loss in the beef cattle population was 3% (Heuer 2014), and the proportion of foetal loss attributed to Hardjo-bovis and/or Pomona sero-positivity (population attributable fraction) was 8.3% (Sanhueza et al. 2013). The simulation used a fix value of 0.03 and 0.083 for the risk of foetal loss and the proportion of foetal losses attributed to *Leptospira* sero-positivity, respectively. A normal distribution of mean NZ$180 and standard deviation of NZ$60 was used to simulate the lost profit for each calf not reared in the population due to leptospirosis (Nicola Schreurs, personal communication, 2016; `www.interest.co.nz/rural`).

A serological survey of beef cattle in New Zealand showed that 98.5% (95% PI 91.9%–99.7%) of herds have at least one cow sero-positive (MAT $\geq$48) to either Hardjo-bovis or Pomona with an average animal sero-prevalence of 65.3% (95% PI 54.8%–74.7%) (Chapter 2). To simulate the level of *Leptospira* sero-positivity in the beef cattle population, a Beta distribution with mode 0.985 (95% lower boundary=0.919) for the herd level prevalence and a Beta distribution with mode 0.653 (95% lower boundary=0.548) for the animal sero-prevalence were used.

The total population of beef breeding cows in New Zealand was 970,000 animals (Anonymous 2015c). Loss from mating to weaning in New Zealand was about 20%, of which 10% occurred from mating to pregnancy diagnosis (McFadden et al. 2005), and the other 10% occurred between pregnancy diagnosis and weaning (Heuer

2009a). Expert opinion suggested a Beta distribution for simulating the replacement rate with a mode 0.20 and a 95% upper boundary of 0.25 (Anne Ridler, personal communication, 2016). A Beta distribution of mode 0.80 and 95% lower boundary of 0.75 was used to simulate the loss from mating to weaning. In summary, about 80% of the breeding cows were assumed to produce offspring and about 20% of them were retained in the herd as replacements.

Sheep In a vaccination trial, Vallée et al. (2014) observed a higher weight gain of 12g/day in vaccinated sheep compared to unvaccinated controls on the same farm in a period of 53 days after tail docking (about 636g heavier on average). A fix value of 0.636kg was used to simulate this live weight gain. The observed difference occurred in 1/8 sheep farms where animals were challenged at a young age. Hence, it was assumed that 12.5% (1/8) of the sero-positive flocks will experience this weight difference in the sheep population (Beta distribution of mode 0.125 and 95% upper limit of 0.471). Dressing percentage was simulated using a Beta distribution of mode 0.42 and 95% upper limit of 0.48 (Nicola Schreurs, personal communication). The value per kilogram of carcass weight was assumed to follow a normal distribution of mean NZ$4.5/Kg and a standard deviation of NZ$1.5/kg (Nicola Schreurs, personal communication, 2016; `www.interest.co.nz/rural`).

Ridler et al. (2015) observed increased odds (OR=13.8) for foetal loss in ewes sero-positive to Pomona compared with sero-negative ewes in the same flock. It was estimated that 75% of the foetal loss in sero-positive animals to Pomona was due to Pomona infection. A fix value of 0.75 was used to simulate foetal loss due to Pomona in Pomona sero-positive ewes. This type of loss associated with Pomona sero-positivity occurred in one of eight farms involved in a vaccination trial (Vallée et al. 2016; Ridler et al. 2015). At the population level, this kind of abortion outbreak due to Pomona was thought to be a rare event (Anne Ridler, personal communication, 2016). Hence, instead of 1/8 (12.5%) we assumed that such Pomona effects would occur on only 1% and not more than 5% of breeding flocks of the New Zealand sheep population. Therefore, a Beta distribution with mode 0.01 and 95% upper boundary of 0.05 was used. A normal distribution of mean NZ$30 and standard deviation of NZ$10 was used to simulate the profit value per lamb (Nicola Schreurs, personal communication, 2016; `www.interest.co.nz/rural`).

Median sero-prevalence to Hardjo-bovis and/or Pomona was 96.7% (95% PI 90.8%–98.9%) at the flock level, and 54.3% (95% PI 44.5%–63.4%) at the animal level (Chapter 2). A Beta distribution of mode 0.967 and 95% lower boundary of 0.908, and a Beta distribution of mode 0.543 and 95% lower boundary of 0.445 were used to simulate Hardjo-bovis and/or Pomona sero-positivity at the flock and animal level, respectively. Median sero-prevalence to Pomona only was 76.1% (95% PI 66.4%–83.6%) at the flock level and 8.7% (95% PI 6.2%–11.4%). To simulate the level of Pomona sero-positivity in the population of ewes, a Beta distribution of mode 0.761 (95% lower limit=0.664), and mode 0.087 (95% upper limit=0.114) were assumed for flock and animal level, respectively.

The total population of ewes in New Zealand was 18,890,000 animals (Anonymous 2015c). The average number of lambs per ewe in New Zealand was reported to be 1.28 (Anonymous 2015a). A fix value of 1.28 was used to simulate this number. To simulate the replacement rate of ewes, expert opinion suggested a Beta distribution of mode 0.30 and 95% upper boundary of 0.40 (Anne Ridler, personal communication, 2016).

Deer Ayanegui-Alcerreca (2006) observed 3.7kg higher slaughter weights in unvaccinated deer sero-negative or without culture and/or urine dark field microscopy evidence of *Leptospira* than unvaccinated deer with evidence of infection on the same farm. Subharat et al. (2012) reported later that vaccinated deer were 6.7kg, 3.1kg and 4.0kg heavier prior to slaughter than unvaccinated animals on three out of four farms enrolled in a randomised clinical trial. Overall, it was observed that vaccinated deer were on average 3kg heavier than unvaccinated deer, suggesting a detrimental effect of infection in unvaccinated deer. To simulate the live weight loss in infected deer, a fix value of 3.0kg was used. The 3kg of weight loss represented an average across herds. Therefore, a Beta distribution of mode 1.00 and 95% lower boundary of 0.90 was assumed to simulate the proportion of affected animals given infection (Peter Wilson, personal communication, 2016). The dressing percentage was simulated using a Beta distribution of mode 0.56 and 95% upper limit of 0.60 (Peter Wilson, personal communication, 2016). The carcass weight price per kilogram was assumed to be normally distributed with mean NZ$7.5/kg and standard deviation of NZ$0.5/kg (Peter Wilson, personal communication, 2016).

In a randomised vaccination trial conducted in four deer farms, Subharat et al. (2011) observed a mean higher weaning percentage of 6% (Range = 2%–10%) in vaccinated hinds against leptospirosis compared with unvaccinated hinds, suggesting that *Leptospira* infection reduced weaning rate. A higher difference of 9% had been observed earlier on one deer farm (Ayanegui-Alcerreca 2006). This loss was modelled by a Beta distribution with a mode of 0.06 and a 95% upper boundary of 0.27 (estimated 95% upper confidence limit of observed weaning difference). Since this was a mean of all sero-positive deer in the herd, the proportion of affected was simulated by a Beta distribution with a mode of 1.00 and 95% lower boundary of 0.90 (Peter Wilson, personal communication, 2016). Expert opinion suggested a normal distribution of mean NZ$270 and standard deviation of NZ$50 for the net profit of selling a weaned deer (Peter Wilson, personal communication, 2016).

The total population of mated hinds in farmed deer herds in New Zealand was 458,100 animals (Anonymous 2014). Based on expert opinion, a Beta distribution with mode 0.80 and 95% lower boundary of 0.70 was used to simulate the percentage of deer calves weaned per hind mated (Peter Wilson, personal communication, 2016). Similarly, the replacement rate of deer herds was simulated by a Beta distribution with a mode of 0.20 and a 95% upper boundary of 0.3 (Peter Wilson, personal communication, 2016).

A serological survey showed that 81.1% (95% CI 72.8%–87.3%) of deer herds were sero-positive to *Leptospira* with an animal prevalence of 60.8% (95% CI 58.7%–62.9%) (Ayanegui-Alcerreca et al. 2010). Herd level prevalence was therefore simulated by a Beta distribution with a mode of 0.811 and 95% lower limit of 0.728, and animal prevalence by a Beta distribution with a mode of 0.608 and a 95% lower limit of 0.587.

Cost of vaccination

Almost all dairy herds (90%) are believed to be vaccinated against leptospirosis in New Zealand (Heuer 2009b), but surveys showed that 18%–25% of beef cattle herds, 5%–9% of deer herds and hardly any sheep flocks (∼1%) were vaccinated (Dreyfus et al. 2011; Wilson et al. 2008; Heuer 2009b). Weighted mean and upper 95% confidence interval were used to simulate vaccination coverage in the popu-

lation of beef cattle (0.214, 95% upper boundary=0.243), deer (0.076, 95% upper boundary=0.107), and sheep (0.006, 95% upper boundary=0.018). A fix value of 0.9 was used to simulate vaccination coverage in dairy cattle.

The value of a vaccine dose used was a fix value of 75 cents per ml, which resulted in a cost of NZ$1.5 for beef cattle and deer, and NZ$1.125 for sheep. Usually, two initial vaccine doses four to six weeks apart, and an annual booster thereafter are required to prevent leptospirosis. Labour costs of vaccination and professional time were not included in the simulation since no estimates were available. Animal vaccination is often performed by the farmer and when performed by a veterinarian, vaccination is likely to be organised within a farm visit programmed for another reason (e.g. animals weighing).

The above mentioned population sizes of breeding animals of beef cattle, deer and sheep were used to estimate vaccination cost. For dairy cattle, a population of five million cows was used. To estimate the cost of vaccination for replacements in each species, the population of females was multiplied by the stated replacement rates. The population of replacements was then multiplied by the proportion of vaccinated animals considering two inoculations (sensitizer and booster) comprising an initial course of vaccination. To estimate the cost of annual booster vaccination of the adult breeding stock, the population of dairy cows, beef cows, hinds and ewes were multiplied by the proportion of vaccinated animals and vaccination cost (annual booster). These two costs (sensitizer-booster and annual booster) were added to estimate the total cost of vaccination in these species.

4.4 Results

4.4.1 Annual number of human leptospirosis cases

The stochastic median of the annual number of mild and severe leptospirosis was 1,567 cases (95% PI 919–2,542). This included 252 cases (95% PI 63–673) with severe signs of the disease which was equivalent to an under-ascertainment of 2.76 (95% PI 0.69–7.37) times for severe disease compared with an average of 91 notifications per year from 2001 to 2014. Table 4.1 shows the expected annual number of leptospirosis cases in abattoir workers, farmers, veterinarians and people working in occupations not at risk of leptospirosis.

Table 4.1: Expected median number of severe and mild leptospirosis cases per year and 95% probability interval (95% PI) in abattoir workers, farmers, veterinarians, and not at risk occupations.

Occupation	**Severe (95% PI)**	**Mild (95% PI)**	**Total (95% PI)**
Abattoir worker	77 (19–210)	397 (205–686)	484 (262–804)
Farmer	120 (26–375)	628 (252–1,290)	766 (314–1,522)
Veterinarian	2 (0–7)	11 (3–26)	13 (4–31)
Not at risk	46 (11–122)	233 (129–392)	284 (166–460)
Total	252 (63–673)	1,288 (715–2,168)	1,567 (919–2,542)

4.4.2 DALYs

Table 4.2 shows stochastic analysis results of DALYs estimation for the entire New Zealand population (n=4,242,048) and stratified by occupations at risk (n=88,674) and not at risk of leptospirosis (n=3,582,076). In terms of DALYs, a median of 0.43 (95% PI 0.05–3.12) per 100,000 people was estimated. However, in the stratum of people working in high risk occupations (abattoir workers, farmers and veterinarians), annual DALYs was 39 times higher (16.76; 95% PI 2.06–122.26 per 100,000).

Table 4.2: Estimated median annual DALYs and 95% probability interval (95% PI) in New Zealand, and stratified by occupations at risk and not at risk of leptospirosis.

Occupation	DALYs (95% PI)	DALYs per 100,000 (95% PI)
At risk[a]	14.86 (1.83–108.41)	16.76 (2.06–122.26)
Not at risk	3.28 (0.40–24.41)	0.09 (0.01–0.68)
Total	18.15 (2.23–132.36)	0.43 (0.05–3.12)

[a] Abattoir workers, farmers and veterinarians.

4.4.3 Human and animal cost of leptospirosis

Table 4.3 summarises simulation results for the cost of infection in humans and animals. The median annual cost of leptospirosis associated with absence from work, treatment of clinical disease in humans, and production loss in beef cattle, sheep, and deer was NZ$16.35 (95% PI 9.06–28.59) million. Adding the cost of vaccination for these species and that of dairy cattle, the total cost of leptospirosis was NZ$25.36 (95% PI 17.99–37.65) million.

The cost of human leptospirosis due to time absent from work and treatment was NZ$4.49 (95% PI 1.55–11.49) million, while production loss in beef cattle, sheep and deer resulted in a total annual cost of NZ$11.31 (95% PI 5.50–22.29) million. At the individual animal level, annual production loss was NZ$12.49 (95% PI 4.45–34.30) per hind, NZ$2.04 (95% PI 0.52–7.38) per beef cow, and NZ$0.14 (95% PI 0.04–0.36) per ewe. The production loss due to leptospirosis could not be estimated for dairy cattle since most herds (90%) are vaccinated against this disease and no investigations were carried in the past 20 years. Vaccination cost in dairy cattle, beef cattle, deer and sheep amounted to NZ$8.99 (95% PI 8.19–10.00) million. Ninety two percent of the total cost of vaccination was attributable to dairy cattle (NZ$8.30 million).

Table 4.3: Annual cost associated with human leptospirosis, production loss due to disease in beef cattle, sheep, and deer, and current vaccination cost to prevent leptospirosis in dairy cattle, beef cattle, sheep, and deer.

Species	**Type of cost**	**Cost (Million NZ$)**	**95% PI**
People	Absence from work	2.56	0.96–5.64
	Treatment	1.85	0.34–6.52
	Subtotal public health	4.49	1.55–11.49
Dairy cattle	Vaccination	8.30	7.55–9.28
Beef cattle	Abortion	0.44	0.15–0.72
	Reduced growth	1.53	0.12–6.70
	Production loss	1.97	0.51–7.15
	Vaccination	0.38	0.32–0.44
	Subtotal beef cattle	2.35	0.88–7.52
	Sheep Abortion	0.58	0.06–2.45
	Reduced growth	1.93	0.26–5.80
	Production loss	2.70	0.68–6.80
	Vaccination	0.22	0.05–0.58
	Subtotal sheep	2.95	0.90–7.05
Deer	Reduced weaning rate	4.15	0.53–14.08
	Reduced growth	1.56	1.16–2.03
	Production loss	5.72	2.04–15.71
	Vaccination	0.07	0.04–0.10
	Subtotal deer	5.79	2.11–15.77
Subtotal	Production loss	11.31	5.50–22.29
Subtotal	Vaccination	8.99	8.19–10.00
Subtotal	Production loss + Vaccination	16.35	9.06–28.59
Total	Public health + Production loss + Vaccination	25.36	17.99–37.65

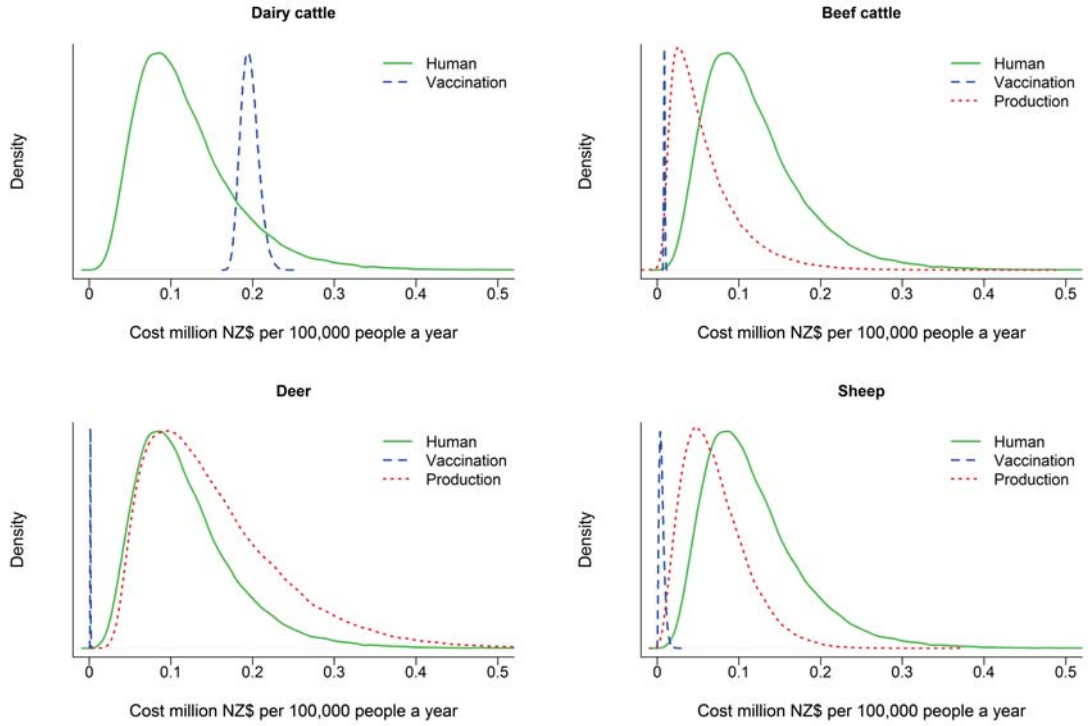

Figure 4.1: Distribution of the annual cost of leptospirosis due to production loss, and vaccination in dairy cattle, beef cattle, deer and sheep per 100,000 people. The distribution of the annual cost of human leptospirosis per 100,000 people is also shown.

Figure 4.1 shows the distribution of the annual cost of leptospirosis in humans (absence from work and treatment), and animals (production loss and vaccination cost) scaled at a rate per 100,000 people. The cost due to absence from work and treatment (NZ$105,720; 95% PI 36,435–270,770 per 100,000) was higher than the cost due to production loss and vaccination in beef cattle (NZ$55,380; 95% PI 20,812–177,350 per 100,000) and sheep (NZ$69,530; 95% PI 21,114–166,280 per 100,000), but lower than the production loss and vaccination cost in deer (NZ$136,390; 95% PI 49,638–371,830 per 100,000) and the cost of vaccination in dairy cattle (NZ$195,660; 95% PI 178,090–218,830 per 100,000). The total production loss in deer (NZ$134,830; 95% PI 48,065–370,450 per 100,000) was 2.9 times higher than in beef cattle (NZ$46,543; 95% PI 12,002–168,650 per 100,000) and 2.1 times higher than in sheep (NZ$63,735; 95% PI 15,905–160,190 per 100,000). On the other hand, the current vaccination expense in deer (NZ$1,527; 95% PI 983–2,239 per 100,000) was 5.8-fold lower than the expense in beef cattle (NZ$8,852; 95% PI 7,501–10,337 per 100,000 people) or 3.4-fold lower than the expense in sheep (NZ$5,141; 95% PI 1,250–13,543 per 100,000 people), and only 0.8% of the cost of

vaccination in dairy cattle.

4.5 Discussion

The aim of this study was to estimate the public health burden and economic cost of leptospirosis in New Zealand. The analysis provided estimates for under-ascertainment, DALYs, and the combined annual median cost of disease in humans, loss of production in animals, and disease prevention (vaccination).

Stochastic simulation results revealed that both severe and mild human leptospirosis cases (1,567; 37 per 100,000) appear to be highly under-ascertained (17-fold) compared with annual notifications. However, the 91 cases (2.2 per 100,000) notified on average between 2001 and 2014 in New Zealand (ESR 2002-2015) are likely to be severe in clinical manifestations. Therefore, a more valid comparison may be based on the 252 severe cases (5.9 per 100,000) predicted by the stochastic simulation, which would result in a 2.8-fold under-ascertainment rate. Costa et al. (2015) conducted a study to estimate the global burden of leptospirosis. They estimated the annual leptospirosis morbidity for Australasia at 9.13 cases per 100,000 and for New Zealand at 3.48 cases per 100,000 (about 148 cases per year). While this is only 1.5-fold above the notified case rate and may be valid for severe cases, it does not account for less severe forms of the disease that are less likely to be notified. Our estimation approach of the annual number of leptospirosis cases incorporated information on the risk of clinical leptospirosis as a proportion of influenza-like illness in the population, the so called "population attributable risk" (PAR). PAR was derived from the observed association between serological evidence of *Leptospira* and the individual recall of influenza-like illness in different occupational populations in New Zealand. Therefore, our incidence estimate reflects the occurrence of disease (severe and mild) due to *Leptospira* infection in the population.

The general formula for DALYs calculation (Formula 4.1) adds disability (YLD) and premature death (YLL). In New Zealand, however, death due to leptospirosis is extremely rare. No deaths attributed to leptospirosis have been reported in notified cases of recent years (ESR 2002-2015). Thus, in the absence of mortality data, DALYs were based on disability only (YLD). Consequently, DALYs for leptospirosis in New Zealand were relatively low (0.43 per 100,000). Torgerson et al. (2015) estimated the global burden of leptospirosis at 41.8 DALYs per 100,000 and for New Zealand at 6.3 DALYs per 100,000. DALYs estimates in this study were mainly driven by YLL, explaining the discrepancy with our country specific DALYs

estimate.

In New Zealand leptospirosis is largely an occupationally acquired disease affecting people in close contact with animals such as abattoir workers, farmers, farm workers or veterinarians (ESR 2002-2015). Consequently, when stratifying the results for people working in high-risk occupations, the estimated DALYs were considerably higher (16.76 per 100,000) than in the rest of the population (0.09 per 100,000). The global disease burden of Torgerson et al. (2015) was 2.5 times higher than the burden we estimated for New Zealand's occupationally at risk population.

Compared with other zoonotic diseases, the DALYs for the occupationally at risk population were lower than the 717 DALYs per 100,000 estimated globally for tuberculosis, the 65 DALYs per 100,000 for cholera, or the 109 DALYs per 100,000 for campylobacteriosis. However, they were similar to the 21 DALYs per 100,000 estimated for rabies and the 12 DALYs per 100,000 estimated for dengue (Murray et al. 2012).

The annual estimated cost associated with days absent from work due to disease and treatment of human leptospirosis was NZ$4.49 million. We assumed a cost of NZ$183 per each day a worker was absent from work (Anonymous 2015b). However, a survey of wellness in the work place (Anonymous 2015d), estimated the average cost of a day off-work for a business in NZ$616, which includes salary cost of absent individuals, replacement costs (i.e. temporary staff or additional overtime) and lost service or production time. If we had used this estimate, the annual cost associated with work absenteeism and treatment would have been NZ$10.64 (95% PI 3.95–24.31) million.

Leptospirosis was reported to cause long term effects in 30.2% of people experiencing acute illness (Goris et al. 2013). These chronically affected individuals may spend a variable amount of time unable to return to their work, and only a proportion of them may be able to continue working in their usual occupation, while others may move into part-time roles or to another job. This is an area that needs to be further researched in New Zealand since there is no clarity about long terms effects of leptospirosis in the population. Although we did include chronic illness for DALYs estimation, we included them only partially in the cost estimation of disease in humans (post-acute illness of 2 months) since there was no reliable information

about off-work pattern of chronically affected people. If we had assumed that all chronically ill people were unable to return to work for the full duration of post-acute illness, then the cost of leptospirosis due to absence from work and treatment would have been NZ$5.63 (95% PI 1.14–33.89) million.

The estimated cost of leptospirosis is lower than for example the cost of campylobacteriosis in New Zealand that was estimated to be NZ$36 million per year (Gadiel and Abelson 2010). However, campylobacteriosis is the most common zoonotic disease in New Zealand with a notified case rate of 150.3 per 100,000 in 2014 compared with 1.2 per 100,000 for leptospirosis in the same year (ESR 2015).

We assumed that all individuals working in occupations at risk of leptospirosis were exposed to the same risk of infection, which may not be the case since different activities within an occupation may put individuals at higher or lower risks of exposure. For example, the infection rate of abattoir workers at the beginning of the slaughter line was higher than for those working in the boning room, chillers or office (Dreyfus et al. 2015a). Similarly, the sero-prevalence of *Leptospira* in farmers of beef, sheep and/or deer was higher when they assisted animals at calving compared to farmers not involved in this activity (Chapter 2). The population burden estimate could be increasingly refined by taking into account different risks of human exposure within an occupation. However, resources required to obtain that level of detail are likely to be high, compromising feasibility and efficiency.

Combining field data on production loss in beef cattle, sheep and deer with serological survey information on the level of Hardjo-bovis and Pomona sero-positivity, and assuming the proportion of affected farms in the population based on production loss data and expert opinion, the annual cost of leptospirosis due to production loss was estimated for these livestock species at NZ$11.31 million. About half of the simulated production loss cost arose from the effects of leptospirosis in deer (Table 4.3). In this species, production loss was characterised by reduced growth of unvaccinated compared to vaccinated deer (Subharat et al., 2012a), and of infected versus non-infected one year old deer (Ayanegui-Alcerreca 2006). In addition, a lower weaning percentage was observed in unvaccinated vs. vaccinated deer, suggesting a detrimental effect of leptospirosis (Subharat et al. 2011; Ayanegui-Alcerreca 2006).

In cattle, the effect of the adapted serovar Hardjo-bovis on foetal loss has been

controversial and comparatively weaker than the effect of Pomona on foetal loss (Elder et al. 1985; Sanhueza et al. 2013). An example of lack of association between Hardjo sero-positivity and abortion is the study conducted by Guitian et al. (1999) in dairy cattle in California. The authors did not observe an association between Hardjo sero-positivity and abortion but they observed an increased time from calving to conception for sero-positive compared to sero-negative cows. In Victoria, Australia, Chappel et al. (1989) observed that Hardjo did not contribute significantly to foetal loss of cattle. Nevertheless, Hardjo is considered an infectious cause of abortions in cattle worldwide (Grooms 2006) and its effect on foetal loss has been observed in experimental (Ellis et al. 1986) and observational studies (Johnson et al. 1974; Slee et al. 1983). In New Zealand, Sanhueza et al. (2013) observed that serology results for both Hardjo-bovis and Pomona were positively associated with foetal loss of beef cattle. Those findings suggested that 8.2% percent of abortions were attributable to Hardjo-bovis or Pomona infection (Sanhueza et al. 2013). In terms of live weight, results from a vaccination trial indicated that vaccinated heifers were 8kg and 14kg, statistically marginally significant (p=0.07) heavier than unvaccinated heifers in two out of seven beef farms (Vallée et al. 2014), which suggested that leptospirosis may have an impact on live weight of cattle.

In sheep, the role of Hardjo-bovis on abortions has not been clearly established in New Zealand, whereas Pomona has been identified as a potential cause of abortion (Ridler et al. 2015; West et al. 2004). Additionally, a temporary higher (p=0.04) daily weight gain of 12g/day during 53 days in one out of eight sheep farms were observed in vaccinated beef cattle and sheep compared to unvaccinated counterparts, respectively (Vallée et al. 2014).

Although production loss due to leptospirosis does not appear to occur in a high proportion of infected beef herds and sheep flocks, they can represent a significant economic loss for the affected farm. The total cost of leptospirosis considering production loss and vaccination expenses was NZ$25.36 million. This estimate is comparatively lower than for example the annual NZ$44.5 million cost estimated for BVD in dairy cattle of New Zealand (Heuer et al. 2007).

Due to lack of information, there were costs not considered in our estimation, like labour cost of vaccination, professional time, diagnosis cost of animal cases, and individual animal disease and outbreak management costs. Therefore, our estimate

of total cost of leptospirosis may be an underestimate of the cost of leptospirosis in New Zealand.

Production loss due to leptospirosis was suggested to be reduced by vaccination but the cost-benefit of this practice may be a limiting factor for the widespread use in beef cattle, sheep and deer. The vaccination cost used in our simulation was NZ$1.5 per vaccinated beef cow and hind, and NZ$1.125 per vaccinated ewe. The comparison with the simulated cost of lost production suggested that the financial benefit may exceed the vaccination cost for hinds, but not for sheep or beef cattle. However, for a small proportion of highly infected herds/flocks that also experienced frequent abortion or poor growth rates, vaccination still appears to be cost-effective. Ultimately, vaccination of any livestock species is currently believed to be the most effective means to reduce human exposure to *Leptospira* and potentially the economic loss due to absence from work and treatment of illness.

4.6 Conclusion

This study quantified the burden and cost of leptospirosis in New Zealand. Findings suggested that the incidence of human leptospirosis is markedly under-ascertained, especially for less severe forms of illness. DALYs indicate the loss of healthy life years, which were particularly high in groups occupationally at risk of leptospirosis. DALYs estimates provided by this study inform livestock industries and human health authorities to prioritise public health interventions. Although potentially underestimated, the estimated NZ$25.36 million cost associated with disease in humans and livestock, quantifies the potential economic gain if disease were prevented.

4.7 Acknowledgement

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Chapter 5

A systematic review and meta-analysis of the efficacy of *Leptospira borgpetersenii* serovar Hardjo and *Leptospira interrogans* serovar Pomona vaccines to prevent urinary shedding in cattle, sheep and deer

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5.1 Summary

Leptospirosis in humans is primarily an occupational disease in New Zealand that affects mostly workers in contact with livestock. Vaccination is not routinely performed in beef cattle, sheep and deer herds/flocks, which all have a high seroprevalence of agglutinating antibodies to *Leptospira*. Vaccination of these livestock species can potentially reduce human exposure and hence the incidence of human illness, if it effectively prevents shedding of leptospires in urine. Several trials have assessed vaccine efficacy in the past but there are no attempts to evaluate these trials jointly, or assess potential causes of vaccine success or failure. This study aimed to identify valid vaccination trials, critically evaluate them, estimate vaccine efficacy to prevent urinary shedding of *Leptospira borgpetersenii* serovar Hardjo (Hardjo) and *Leptospira interrogans* serovar Pomona (Pomona), and explore causes for heterogeneity between trials. A systematic review and meta-analysis of vaccination trials conducted in cattle, sheep, and deer was performed. Literature evaluating commercial vaccines to prevent urinary shedding of leptospires after "artificial" conjunctival or "natural" challenge, and published from 1980 to 2015 were selected. Three databases (Web of Science, Scopus, and PubMed) were used to search for relevant articles. Additional sources of trials were also searched. Heterogeneity of trial effects was assessed using Q and I^2 statistics. Publication bias was assessed visually using funnel plots and statistically using the Egger test for culture results only. The "Trim and Fill" procedure was also used to assess the extent of publication bias in the pooled estimate. 1237 articles were initially identified (1233 through database search and four through additional sources). After title and abstract screening, critical appraisal, and data extraction; ten, five, and four trials assessing Hardjo urinary shedding by bacteriological culture, polymerase chain reaction (PCR), and fluorescent antibody (FA), respectively, were included in three separate random effects meta-analyses. Only one trial assessed urinary shedding by dark field microscopy (DFM). No trials assessing vaccine efficacy against Pomona challenge fulfilled the requirements to be included in the meta-analyses. Vaccine efficacy to prevent shedding of leptospires in urine of cattle or deer after Hardjo natural or artificial challenge was evaluated in the trials included in the meta-analysis. Vaccine efficacy was estimated to be 82.1% (95% CI 70.8%–89.0%) when urinary shedding was assessed by culture in animals without evidence of infection at challenge. While there was no evidence of heterogeneity of study effects (p=0.49), trials using monovalent vaccine exhibited a marginally higher efficacy (p=0.06) than trials using vaccines with $\geq$2 serovars.

Significant evidence of publication bias (p=0.03) was present in favour of small trials showing strong protective effects. When shedding was assessed by PCR or FA; significant evidence of heterogeneity of study effects (p<0.1, I^2 >50) was calculated. The estimated vaccine efficacy to prevent shedding of Hardjo in urine may be sufficient to achieve herd immunity. Nevertheless, the preventive effect of vaccines against shedding after Pomona challenge in livestock species should be assessed. Further trials should also consider evaluating the apparent protective effect of monovalent vaccines v/s vaccines with ≥2 serovars; particularly under the same farm conditions.

5.2 Introduction

The mean incidence of notified human leptospirosis in New Zealand is 2.2 cases per 100,000 people (ESR 2002-2015), one of the highest among developed countries (Pappas et al. 2008). Infection with *Leptospira* in humans can cause a severe life threatening disease but more often it causes a mild, self-limited influenza-like disease (Haake and Levett 2015). The most common serovars in notified cases in New Zealand are *Leptospira borgpetersenii* serovar Hardjo (Hardjo), *Leptospira interrogans* serovar Pomona (Pomona), *Leptospira borgpetersenii* serovar Ballum (Ballum), *Leptospira interrogans* serovar Copenhageni (Copenhageni) and *Leptospira borgpetersenii* serovar Tarassovi (Tarassovi). People occupationally in contact with animals and/or their environment are at higher risk of *Leptospira* exposure. Approximately 80% of the notified cases with a recorded occupation were farm or abattoir workers (ESR 2002-2015). A serological survey of abattoir workers estimated the prevalence of Hardjo and/or Pomona as 10.9% with a 95% confidence interval (95% CI) of 8.5%–13.9% (Dreyfus et al. 2014). In farmers and veterinarians, we estimated the sero-prevalence of Hardjo, Pomona, Ballum, Copenhageni and Tarassovi to be 6.6% (95% CI 3.6%–10.9%) and 5.1% (95% CI 2.8%–8.3%), respectively (Sanhueza et al. (2015) and Chapter 2).

Hardjo and Pomona are endemic in beef cattle, sheep and deer herds/flocks in New Zealand. The individual farm serological status can vary from year to year (Subharat et al. 2012b). Different studies have estimated the farm level sero-prevalence to range from 53% - 97%, 64% - 97%, and 70% - 81% on beef cattle, sheep and deer farms, respectively (Blackmore et al. 1982; Ayanegui-Alcerreca et al. 2010; Dreyfus et al. 2011; Subharat et al. 2012b). Furthermore, individual animal sero-prevalence in those farms ranged from 50% - 58%, 21% - 50%, and 34% - 61% in cattle, sheep and deer, respectively. This indicates a high risk of exposure for people in contact with sero-positive animals, especially if we consider that up to 41% (95% CI 30–54) of sero-positive cattle and sheep have been observed shedding leptospires in urine as measured by PCR (Fang et al. 2015).

Evidence suggests that a vaccination programme considering annual vaccination of animals is an effective way to reduce the risk of infection in livestock and transmission to humans. In New Zealand, a reduction in the number of human notified cases coincided with implementation of vaccination in dairy cattle (Marshall 1987).

Currently, vaccination of dairy herds is thought to be approximately 90% but reports suggest up to 23% of beef cattle herds, 1% of sheep flocks, and 9% of deer herds its use is still limited (Wilson et al. (2008), Dreyfus et al. (2011), and Chapter 2).

Several studies have been conducted to evaluate the prevention of urinary shedding of leptospires after artificial or natural challenge in vaccinated animals compared with unvaccinated controls. Early vaccination trials mostly evaluated the effect of experimental Pomona vaccines to prevent infection in cattle after artificial Pomona challenge. In those trials, urinary shedding was measured by culture or dark field microscopy (DFM) and 100% vaccine efficacy was observed in some trials (Hoag and Bell 1955; Webster and Reynolds 1955; Rhodes 1960). However, lower vaccine efficacies were also reported (Gillespie and Kenzy 1958a; Gillespie and Kenzy 1958b; Kiesel and Dacres 1959). It was not until the 1970s that the attention changed to evaluating the efficacy of Hardjo vaccines, with results ranging from 0% - 100% prevention of renal colonisation or shedding of leptospires in urine of cattle challenged with Hardjo (Strother 1974; Tripathy et al. 1976; Bolin et al. 1991).

Urinary shedding of leptospires can be assessed by different methods that vary in their ability to detect the organism in urine. Bacteriological culture has been widely used in the past and whereas the specificity of culture for detecting leptospires may be 100%, its sensitivity appears to be low compared with fluorescent antibody (FA) and polymerase chain reaction (PCR) (Bolin et al. 1989b; Zuerner et al. 2011; Ellis 2015). Nevertheless, bacteriological culture of leptospires is still commonly used in vaccination trials, although not without controversy when it is the only method used for assessing shedding of leptospires in urine (Alt et al. 2012; Rinehart et al. 2012a).

There are several factors that may influence vaccine and vaccination programme efficacy. For example, it is commonly accepted that monovalent vaccines have a higher efficacy than multivalent vaccines in the activation of the immune system and prevention of shedding of leptospires in urine (Brown et al. 2003; Ellis 2015). Age at first vaccination may also be an important factor to consider, especially under farming conditions, since vaccination is less efficient for reducing urinary shedding in already infected compared with naïve animals (Hancock et al. 1984). Moreover, in a pilot study of 44 routinely vaccinated (>5 years) dairy herds, Wilson et al. (2013) observed that the risk of urinary shedding was significantly lower when calves were

vaccinated at <3 months of age compared with animals vaccinated after 6 months of age.

No formal comprehensive assessment of the efficacy of *Leptospira* vaccines to prevent urinary shedding and factors influencing efficacy have been reported, despite that vaccine efficacy is an important factor for vaccination programme effectiveness. This systematic review and meta-analysis aims to estimate the efficacy of Hardjo and/or Pomona vaccines to prevent urinary shedding of leptospires in cattle, sheep and/or deer, and to explore factors that may influence vaccine efficacy.

5.3 Materials and Methods

The review process and reporting of results were guided by the preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement (Moher et al. 2009).

5.3.1 Research question

The literature search aimed to identify articles reporting upon the efficacy of vaccines containing Hardjo and/or Pomona to prevent urinary shedding of leptospires in cattle, sheep and deer to enable estimation of vaccine efficacy in these species.

5.3.2 Literature search strategy

An electronic literature search was conducted to include papers published up to February, 2015. Article databases searched were the Web of Science (including Science Citation Index Expanded and Conference Proceedings Citation Index- Science, Biological Abstracts, CABI: CAB Abstracts, Current Contents Connect, PubMed (MEDLINE), SciELO citation index), Scopus and PubMed.

Key-words used in the search to select relevant studies were: [Lepto* or Weil] and [cattle OR bovine OR cow OR calves OR deer OR cervine OR fawn OR sheep OR ovine OR ewe OR lamb] and [Vacc* or Immun*] and [efficacy OR effect* OR protect* OR shed*]. The asterisk is used to extend the search to related words with similar meaning, e.g. Vacc* searches for vaccine, vaccination and vaccinate. Secondary sources of potentially useful studies were PhD theses available at the Massey University library and the book "*Leptospira* and leptospirosis" (Adler 2015; Ellis 2015).

5.3.3 Screening of records

Article titles and abstracts were screened to select those that evaluated the effects of *Leptospira* vaccination in cattle, sheep or deer. This selection included studies that evaluated antibody response with or without leptospiral challenge and with, or

without urinary shedding assessment. Whenever a title/abstract was inconclusive for the decision to exclude a publication, the article was considered for full-text evaluation.

5.3.4 Eligibility criteria

Full-text articles were reviewed for eligibility. Inclusion criteria used to select articles were: articles containing original information (not reviews); articles evaluating vaccine efficacy in a vaccination trial (either under "natural" exposure or "artificial" conjunctival challenge); articles evaluating shedding of leptospires in urine as an outcome measured by culture, fluorescent antibody (FA), dark field microscopy (DFM) and/or polymerase chain reaction (PCR); and articles using commercially available vaccines published from 1980 to 2015. No language restriction was applied.

5.3.5 Data extraction

Information extracted for each independent trial was title, authors, study type (controlled trial or field trial), species used, vaccine information, age at vaccination, time from vaccination to challenge, challenge method (artificial or natural), serovar used in challenge, challenge dose, route used for artificial challenge, method of shedding assessment (culture, DFM, FA and/or PCR), number of shedders in vaccinated and control groups, and total numbers in vaccinated and control groups. Relevant information from each article was summarised in an Excel spreadsheet. If required, authors were contacted for additional information. Details of all selected trials for the meta-analyses are listed in Appendix VIII: Summary of trials for meta-analysis.

5.3.6 Bias assessment for individual studies

Eligible articles were reviewed for evidence of bias using the tool for assessing the risk of bias in randomised trials described by Higgins et al. (2011). Bias domains assessed in the included articles were: random sequence generation; allocation concealment; blinding of outcome assessment; incomplete outcome data; and selective reporting. Each of these domains were categorised into "low", "high", or "unclear"

evidence of bias (Table 5.1).

5.3.7 Methods for measuring shedding of leptospires in urine

The most common methods for identifying leptospires in urine were PCR, FA, DFM and/or culture. The ability of each test to detect *Leptospira* in urine was expected to be different. This may be particularly relevant for culture due to its lower sensitivity compared with PCR or FA (Bolin et al. 1989b; Zuerner et al. 2011). Therefore, overall measures of vaccine efficacy were estimated independently for each of the tests used for detecting leptospires in urine.

5.3.8 Meta-analysis

Selected articles were included in a random effects meta-analysis using the restricted maximum likelihood (REML) estimator (Viechtbauer, 2005). The inverse variance method was used to assign weights to studies for estimating the overall (pooled) effect. When a study reported results of independent subgroups, for example when treated and control groups were from the same farm and the study reported results of several farms, then each subgroup was treated as an independent trial in the analysis (Borenstein et al. 2009). For each trial, the natural logarithm and the variance of the relative risk (RR = risk of shedding in vaccinated animals divided by the risk of shedding in unvaccinated animals) was estimated. A continuity correction of 0.5 was added to treatment arms with zero cell frequencies in the 2 by 2 contingency table. When the number of vaccinated and unvaccinated animals shedding leptospires in urine was reported at different points in time during a trial, a single composite effect size and variance was estimated (Borenstein et al. 2009). Vaccine efficacy was estimated using the formula 1: $(1 - RR) \times 100$. Overall point RR estimate (pooled RR), 95% confidence interval (95% CI) and 95% prediction interval were reported. The difference between these two intervals is that the 95% CI reflects the accuracy of the mean estimate in the meta-analysis, mainly driven by the number of trials, whereas the 95% prediction interval also takes into account the variance between trial results reflecting the distribution of true effects around the mean estimate (Borenstein et al. 2009).

Q statistic was used to assess heterogeneity of trial results. Significance was evaluated at the alpha-level of 0.1. Heterogeneity was also quantified using I^2 statistic; values <25%, 25% to 75%, and >75% were considered as indicative of low, medium, and high heterogeneity, respectively (Higgins et al. 2003).

Publication bias was assessed only when the number of studies included in the meta-analysis was at least 10 (Higgins and Green 2011). This assessment was done visually using a funnel plot and statistically using the Egger's test (Egger et al. 1997; Sterne and Egger 2005). The "Trim and Fill" method was used to assess the effect of the potential publication bias on the pooled vaccine efficacy estimate (Duval and Tweedie 2000a; Duval and Tweedie 2000b).

A sensitivity analysis was conducted to evaluate the influence of individual trials on the pooled estimate and heterogeneity by fitting several models and leaving one study out each time. The variability of pooled efficacy estimates in each of these partial analyses around the composite estimate was used as a measure of trial influence. Meta-regression was used to explore causes of heterogeneity and to assess differences on vaccine efficacy for levels of moderator variables included in the model.

5.3.9 Software

Analyses were done using R version 3.2.1 (R Core Team 2015), and the R package "metafor" (Viechtbauer 2010).

5.4 Results

5.4.1 Selection of articles

The number of articles screened, excluded and included in the meta-analyses is shown in Figure 5.1. The initial search of key-words identified 1237 titles after deduplication. Once title and abstract screening was performed, 85 records were considered for full-text evaluation.

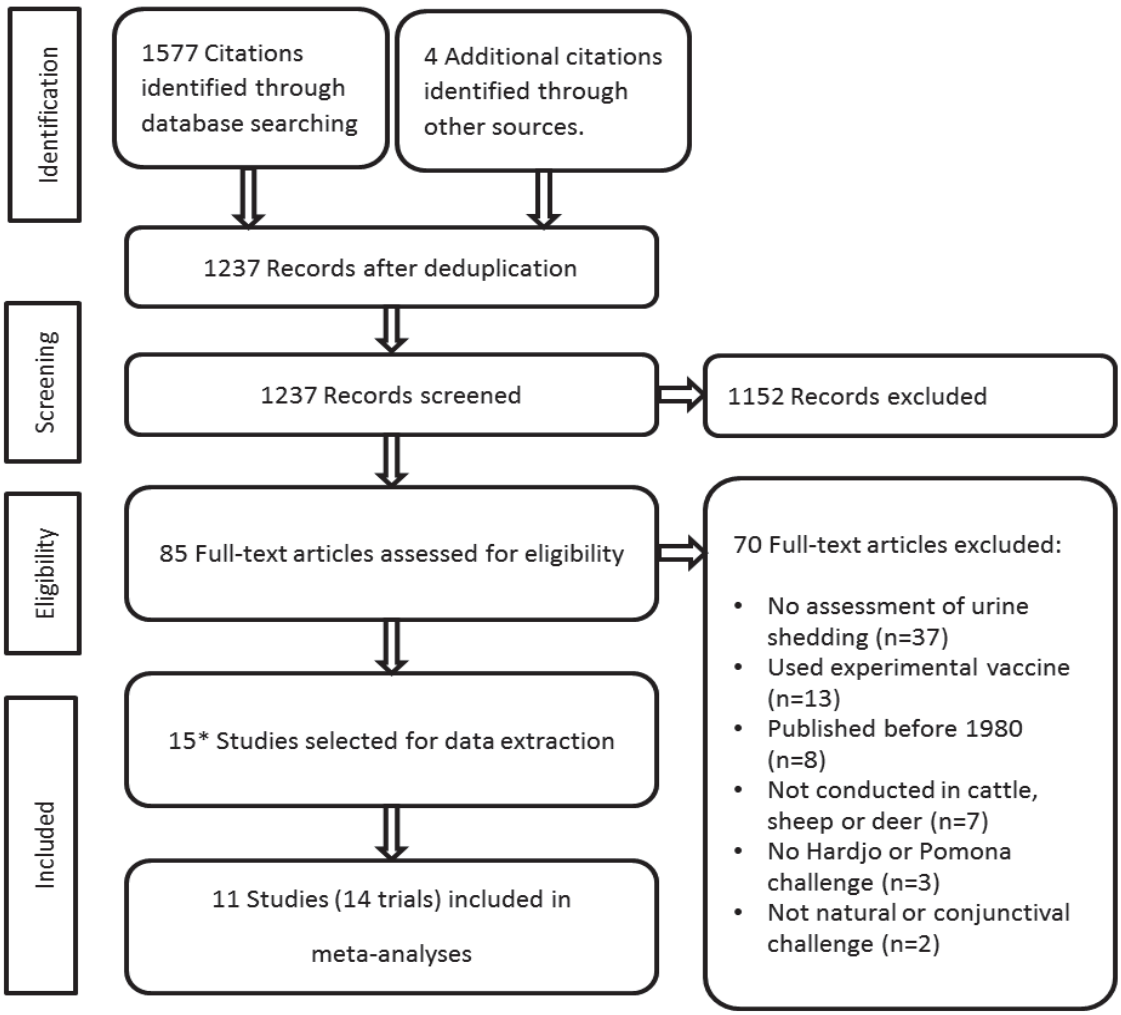

Figure 5.1: A flow chart of systematic selection of studies used in the meta-analyses based on that of Moher et al. (2009). *Four further studies were not used in the meta-analysis (see Meta-analysis exclusions for details).

5.4.2 Data extraction and bias assessment

Results of the bias assessment are presented in Table 5.1. Fifteen articles were reviewed for data extraction and assessment of systematic bias in randomised trials (Higgins et al. 2011). Even though all studies used random allocation of animals, the method used was not always clearly described and was therefore classified as "unclear" risk. Allocation concealment refers to the efforts made to avoid people who were enrolling participants being able to see the allocation sequence (concealment of random sequence) until treatment allocation. This was not clearly described in any of the reviewed articles and was therefore classified as "unclear" risk for all articles. Nevertheless, all studies with "low" or "unclear" risk of bias were potentially used in the meta-analyses, if urinary shedding information was available.

5.4.3 Meta-analysis exclusions

One article (Hancock et al. 1984) was excluded from the meta-analysis because it presented results from a follow-up of animals used in a previous vaccination trial (Allen et al. 1982). The study conducted by Ayanegui-Alcerreca (2006) was also excluded because there was serological evidence of exposure (potentially on-going infection) in the herds at the time of vaccination. The studies conducted by Goddard et al. (1986) and Bolin and Alt (2001) were excluded since the former reduced the vaccine dose by deliberate dilution, and in the latter it was not possible to distinguish culture from FA results. Finally, culture and FA results presented by Ellis et al. (2000) were not used since it was not possible to differentiate urine and kidney culture results.

Table 5.1: Systematic bias assessment of 15 individual studies according to guidelines for randomised trials described by Higgins et al. (2011).

Author (year) of Study	**Random sequence generation**	**Allocation concealment**	**Blinding of outcome assessment**	**Incomplete outcome data**	**Selective outcome reporting**
Cortese et al. (2014)	Unclear	Unclear	Low	Low	Low
Plunkett et al. (2013)	Low	Unclear	Unclear	Low	Low
Zimmerman et al. (2013)	Unclear	Unclear	Low	Low	Low
Rinehart et al. (2012b)	Low	Unclear	Low	Low	Low
Subharat et al. (2012a)	Unclear	Unclear	Unclear	Low	Low
Zuerner et al. (2011)	Unclear	Unclear	Unclear	Low	Low
Ayanegui-Alcerreca (2006)	Unclear	Unclear	Unclear	Low	Low
Bolin and Alt (2001)	Unclear	Unclear	Unclear	Unclear	Low
Ellis et al. (2000)	Unclear	Unclear	Unclear	Low	Low
Bolin et al. (1989b)	Unclear	Unclear	Unclear	Low	Low
Goddard et al. (1986)	Unclear	Unclear	Unclear	Low	Low
Broughton et al. (1984)	Unclear	Unclear	Unclear	Low	Low
Hancock et al. (1984)	Unclear	Unclear	Low	Low	Low
Allen et al. (1982)	Unclear	Unclear	Low	Low	Low
Mackintosh et al. (1980)	Unclear	Unclear	Unclear	Unclear	Low

5.4.4 Articles included in meta-analyses

After the exclusion of articles described above, 11 publications that contained information about 14 independent vaccine trials were included in the meta-analyses. Overall, 12 trials used cattle and two trials used deer. No trial assessed the effect of vaccination to prevent urinary shedding after Hardjo or Pomona challenge in sheep, or Pomona challenge in cattle or deer. Artificial conjunctival challenge with Hardjo was used in seven trials and natural challenge was used in seven field trials. All trials used Hardjo challenge (either artificial or natural). Monovalent vaccines were used in five trials and vaccines with $\geq$2 serovars in nine trials. Appendix VIII: Summary of trials for meta-analysis contains the extracted numbers of vaccinated and control animals used in the meta-analysis plus detailed information on other characteristics reported in each trial.

5.4.5 Meta-analysis of vaccine efficacy assessed by culture

Ten studies were jointly analysed to estimate the effect of vaccination on urinary shedding as determined by bacteriological culture in animals without evidence of infection at vaccination. The estimated relative risk of shedding leptospires in urine of vaccinated versus unvaccinated animals was 0.179 (vaccine efficacy=82.1%; 95% CI 70.8%–89.0%). Figure 5.2 shows a forest plot of individual trial effect sizes and pooled relative risk. The study conducted by Allen et al. (1982) reported urinary shedding at 18 and 22 weeks after vaccination (Appendix VIII: Summary of trials for meta-analysis). A single composite effect size and variance for these two time periods was estimated prior to inclusion in the meta-analysis (Borenstein et al. 2009).

No statistically significant evidence of heterogeneity was observed among effect sizes of individual trials (p=0.49 and I^2=0.0%). However, the risk ratio of shedding leptospires in urine of vaccinated versus unvaccinated animals was 7.0 (95% CI 1.0–51.7) times higher when a vaccine containing $\geq$2 serovars was used compared with a monovalent vaccine (p=0.06). This reflects the higher vaccine efficacy observed in the two trials that assessed the effect of monovalent vaccines on urinary shedding prevention (97.1%) compared with the eight trials that assessed the effect of vaccines with $\geq$2 serovars (79.7%).

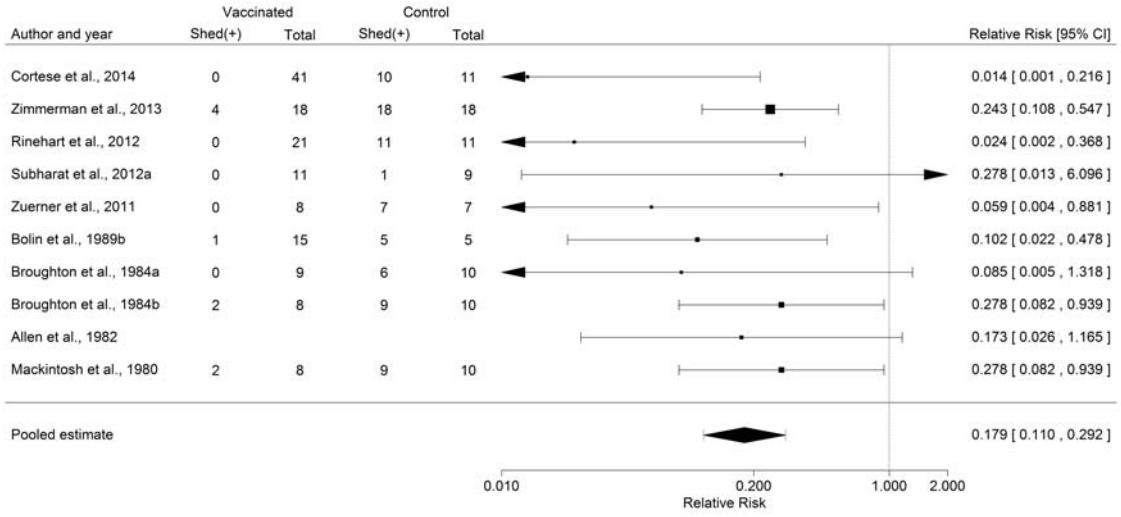

Figure 5.2: Forest plot of 10 trials included in the meta-analysis assessing the effect of vaccination to prevent shedding of leptospires in urine measured by bacteriological culture. The diamond represents pooled mean effect and 95% confidence interval. Vertical bars in the extremes of the diamond represent the 95% prediction interval (0.110–0.292).

Vaccine efficacy did not differ significantly (p=0.26) between trials using artificial challenge and trials using natural challenge, or between trials using cattle or deer (p=0.78).

No important changes in the pooled estimate or heterogeneity were observed in the sensitivity analysis.

Evidence of publication bias (Egger's test p=0.03) was observed in the funnel plot (Figure 5.3) as studies with smaller sample size (larger standard error of the RR) showed comparatively smaller estimates of RR. The asymmetry in the funnel plot was mainly caused by three trials at the left of the adjusted pooled estimate that showed relatively large standard errors and low relative risks. These three trials were conducted under artificial conjunctival challenge conditions in cattle. If we assume that publication bias was the cause of funnel plot asymmetry, then vaccine efficacy would be 78.1% (95% CI 65.2%–86.3%) after adjustment using the "Trim and Fill" method (Duval and Tweedie 2000a; Duval and Tweedie 2000b).

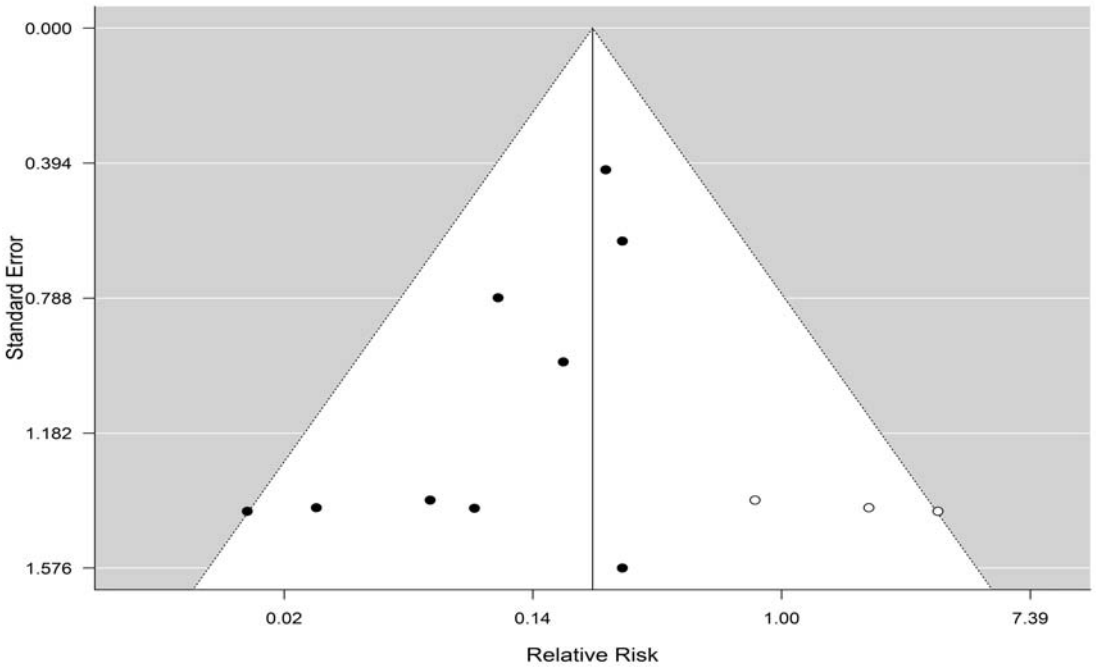

Figure 5.3: Funnel plot illustrating potential publication bias in the data. Studies included in the meta-analysis of culture results are represented by black dots, while hypothetical missing studies identified by the "Trim and Fill" procedure are represented by white dots. The vertical line dividing the funnel represents the adjusted relative risk (RR_{adj}=0.219).

5.4.6 Meta-analysis of vaccine efficacy assessed by PCR

Five studies were included in the meta-analysis of vaccine efficacy against urinary shedding as evaluated by PCR. Significant evidence of heterogeneity of effect sizes was observed (p=0.06; I^2=54.1%). Overall vaccine efficacy was estimated to be 74.8% but the heterogeneity among trial results made this estimate highly unreliable as reflected by the wide 95% prediction interval of the pooled RR (Figure 5.4).

Heterogeneity was reduced (p=0.3; I^2=35.9%) after the inclusion of the variable "time from vaccination to challenge" ($\leq$6 months versus >6 months) in the meta-regression model when PCR was used to detect urinary shedding. The relative risk of shedding in vaccinated compared with control animals was 5.2 (95% CI 0.7–41.2) times higher when time from vaccination to challenge was >6 months (vaccine efficacy=51.8%) compared with $\leq$6 months (vaccine efficacy=90.7%). However, this difference was not statistically significant (p=0.12).

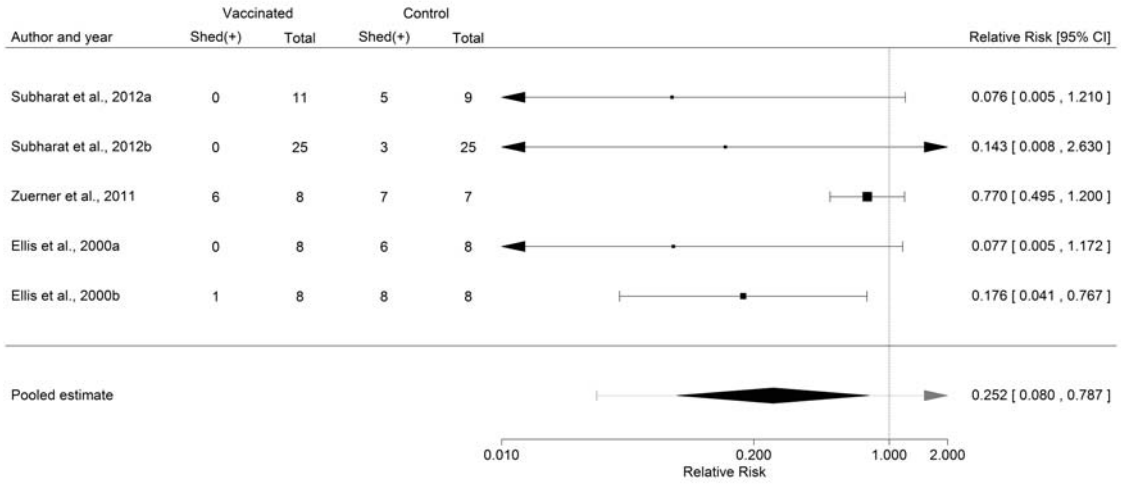

Figure 5.4: Forest plot of five studies included in the meta-analysis assessing the effect of vaccination against urine shedding of *Leptospira* measured by PCR. The diamond represents the pooled mean effect and 95% confidence interval. The dotted grey line extending from the extremes of the diamond represents the 95% prediction interval (0.03–2.04).

When the result of the study conducted by Zuerner et al. (2011) was excluded from the analysis, vaccine efficacy was estimated in 86.8% (95% CI 60.9%–95.6%) and heterogeneity became statistically non-significant (p=0.93; I^2=0%).

5.4.7 Meta-analysis of vaccine efficacy assessed by FA

Four trials evaluated urinary shedding in vaccinated and control animals by FA test. Pooled vaccine efficacy was estimated to be 61.1% (95% CI 0.0%–86.8%). The upper 95% CI of the RR overlapped the value of one and therefore the lower 95% CI of vaccine efficacy was truncated to 0.0%. There was also statistically significant evidence of heterogeneity between trials (p=0.02 and I^2=69.0%) visualised by the large 95% prediction interval in Figure 5.5. The inclusion of "age at vaccination" variable in the meta-regression model reduced heterogeneity marginally (p=0.1; I^2=57.2%). The relative risk of shedding leptospires in urine of vaccinated versus control animals was 7.0 (95% CI 0.6–83.1) times higher in one trial that vaccinated animals at >4 months of age compared to the trials that vaccinated animals at ≤4 months old (p=0.13).

Heterogeneity was no longer significant (p=0.21, and I^2=36.4%) after the removal of the trial conducted by Bolin et al. (1989b) and vaccine efficacy was 76.2% (95% CI 23.8%–92.5%).

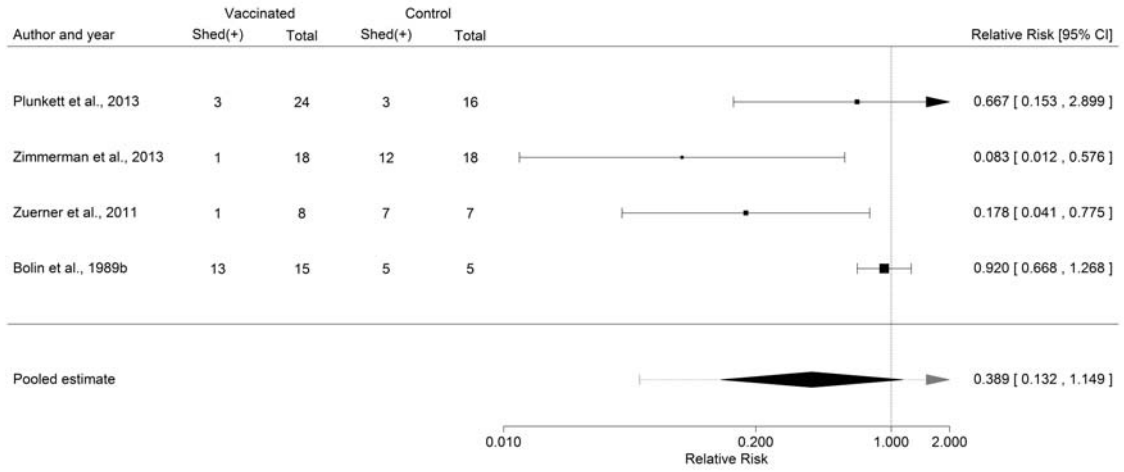

Figure 5.5: Forest plot of four studies included in the meta-analysis assessing the effect of vaccination against urinary shedding of *Leptospira* measured by FA. The diamond represents the pooled mean effect and 95% confidence interval. The dotted grey line extending from the extremes of the diamond represents the 95% prediction interval (0.05–3.01).

5.4.8 Urinary shedding assessed by DFM

One trial evaluated urinary shedding of leptospires in vaccinated and control animals by DFM. This was a field trial in which cattle were challenged by natural Hardjo exposure (Allen et al. 1982). Vaccine efficacy was 77.8% (95% CI 62.6%–86.8%).

5.5 Discussion

Vaccine efficacy to prevent shedding of Hardjo in urine, as measured by culture, was 82.1% (95% CI 70.8%–89.0%). Vaccine efficacy estimates were lower for PCR (74.8%), FA (61.1%) than that observed for culture but the medium percentage of heterogeneity observed (I^2=50%–75%) among individual trials included in the meta-analyses made these estimates highly unreliable (wide 95% prediction intervals).

Bacteriological culture is a highly specific method but it lacks sensitivity to detect leptospires (Ellis 2015), especially when the number of organisms shed in urine is low (Smith et al. 1994). Essential questions are the minimum infectious dose of different *Leptospira* serovars for humans and animals, and whether the number of leptospires shed in urine of some vaccinated animals is below this minimum. Urine culture results of previously unexposed vaccinated cattle under natural challenge conditions have shown transient isolations of leptospires compared with a consistent pattern in unvaccinated controls (Mackintosh et al. 1980; Allen et al. 1982), which may suggest that vaccinated animals shed a lower number of viable leptospires compared than non-vaccinates. The potential presence of antibodies or growth inhibitors were also suggested as a potential causes of culture failure (Smith et al. 1994). Despite this, bacteriological culture was the most frequently used method to assess urine shedding in vaccination trials included in the meta-analysis (n=10), followed by PCR (n=5), FA (n=4) and DFM (n=1). Most trials used more than one method to assess shedding (n=8), while five used culture only and one FA only. Often, disagreement between culture and results from more sensitive methods (e.g. PCR or FA) can be observed in some of the vaccination trials that used more than one method to detect leptospires in urine (Bolin et al. 1989a; Bolin et al. 1989b; Zuerner et al. 2011). Because of those discrepancies and in an effort to reduce heterogeneity in the data, we decided to stratify the meta-analysis by the method used to assess shedding of leptospires in urine. However, the low number of trials available and the extent of heterogeneity due to trial results differences caused very large variation around mean vaccine efficacy estimates from the strata PCR and FA.

Controversy exists for the use of bacteriological culture as the only method to assess vaccine efficacy since its lack of sensitivity may result in an overestimate of vaccine efficacy (Alt et al. 2012), particularly if vaccinated animals shed low number

of leptospires in urine that are not detected by culture. Nevertheless, the clinical and epidemiological significance of a positive urine test result obtained using a more sensitive method (e.g. PCR or FA) remains unclear since those tests may detect dead or non-viable leptospires.

The 82.1% vaccine efficacy estimate could be considered an "optimistic" result due to the lack of sensitivity of bacteriological culture. Nevertheless, it potentially reflects the effect of vaccination on reducing the number of viable leptospires shed in urine. It may be possible that this is enough to achieve herd immunity in the context of a herd vaccination programme involving annual immunisation of animals. Under this condition, vaccination may be still capable of reducing within-herd transmission of *Leptospira*, even if some vaccinated animals shed low numbers of leptospires in urine not detected by culture. In addition, it has to be considered that the occurrence of natural challenge in susceptible animals from vaccinated herds would be diminished; reducing the chance of animal infection and human exposure.

Some experiments showed that multivalent vaccines did not protect cattle against Hardjo challenge when compared with unvaccinated controls as measured by FA (Bolin et al. 1989a; Bolin et al. 1989b). It was observed that a monovalent Hardjo vaccine produced a stronger cell-mediated immune response than a multivalent vaccine (Brown et al. 2003). Meta-regression of urine culture results showed a marginally non-significant (p=0.06) higher Hardjo shedding prevention in trials using monovalent Hardjo vaccines compared with trials using Hardjo vaccines with one or more additional serovars. Although only 2/10 of the trials included in our meta-analysis assessed the effect of monovalent vaccines on shedding prevention, the relatively higher vaccine efficacy observed (97.1%) compared with vaccines containing $\geq$2 serovars (79.7%) suggest that monovalent vaccines may indeed confer a higher protection to animals. More trials using monovalent Hardjo vaccines, particularly under farm conditions and natural challenge, may have to be performed to provide more conclusive data about a possible superiority of mono- versus multivalent vaccines.

Age at vaccination may be an important variable influencing vaccine efficacy in livestock under an endemic natural challenge situation since the cumulative probability of infection increases with time at risk of exposure and vaccination is less effective for reducing urinary shedding of leptospires when animals were already

infected (Hancock et al. 1984). The importance of age at vaccination was hypothesised from a pilot study of vaccinated dairy herds that found a lower prevalence of urinary shedders in herds that vaccinated animals at the age of 3 months or younger compared to herds that started vaccinating at an older age (Wilson et al. 2013). Thus, a lower vaccine efficacy was also expected for trials conducted under natural challenge conditions compared with artificial challenge trials because some animals could have been already infected by the time of vaccination. Although no significant difference in vaccine efficacy was observed in any of the meta-regression models for young animals ($\leq$4 months) compared with older animals (>4months) or between natural and artificial challenge, it is likely that the efforts made by researchers in vaccination trials to include only unexposed or treated animals against *Leptospira*, reduced the chance to observe a different vaccine efficacy across different ages or method of challenge.

Publication bias was evaluated for the meta-analysis of vaccine efficacy assessed by culture since a sufficient number of trials were available (Higgins and Green 2011). For visual assessment of the funnel plot (Figure 5.3), a variety of choices for the vertical axis are available (i.e. standard error, 1/standard error, variance, sample size). For binary outcomes, Sterne and Egger (2001) observed that the standard error is generally preferable for the visual assessment of the symmetry of a funnel plot (Figure 3). The asymmetry in the base of the funnel plot suggested an over-representation of trials reporting strong preventive effects (RR<0.06) when conducted with relatively few animals (large standard error). The "Trim and Fill" procedure (Duval and Tweedie 2000a; Duval and Tweedie 2000a) identified three trials with extreme results in the funnel plot. If we assume that publication bias was the cause of funnel plot asymmetry, then the true vaccine efficacy was lower than the 82.1% estimated in the meta-analysis for culture results. Nevertheless, the 4.0% difference due to potentially missing studies is small and unlikely to have practical implications on herd immunity.

In most reviewed articles there was insufficient information about trial design and methods required for assessing bias in the conduct of clinical trials according to available guidelines (Higgins et al. 2011). The risk of bias in those studies was classified as "unclear". This was especially evident for the design criteria related to random sequence generation, allocation concealment, and blinding of outcome assessment (Table 5.1). Although the inclusion of studies with "unclear" risk of bias

might have influenced the results of the meta-analysis, limiting the meta-analysis only to studies with "low" risk of bias would have left too few studies for analysis. Guidelines for strengthening the report of clinical trials (Schulz et al. 2010) and observational studies (von Elm et al. 2007) are available.

Our meta-analyses were limited to trials evaluating the effect of commercial vaccines on shedding of leptospires in urine of beef, sheep or deer after natural challenge or artificial conjunctival challenge with Hardjo or Pomona that were published from 1980 to 2015. The efficacy of leptospiral vaccines to prevent urinary shedding in sheep after Hardjo challenge, and in ruminant livestock species after Pomona challenge, have not been assessed recently. Although articles published earlier than 1980 evaluated these effects, they were mainly conducted using experimental vaccines and "unnatural" challenge routes (intraperitoneal, subcutaneous, intramuscular, and intravenous). Exceptions were the trials conducted by Gillespie and Kenzy (1958a), Gillespie and Kenzy (1958b), and Kiesel and Dacres (1959). These trials assessed the efficacy of monovalent Pomona vaccines to prevent shedding of leptospires in urine of cattle (by culture or DFM) after artificial conjunctival challenge with Pomona. Results showed vaccine efficacies of 100%, 100%, 58.3%, 33.3% and 0% in five independent trials reported by those authors. The reason for limiting the time frame for inclusion of studies to the last 35 years was to reduce potential differences in vaccine quality, challenge methodology and study design standards.

A common approach for synthesizing study results in a meta-analysis is the inverse variance method. This methodology allows for fixed or random effects estimation of summary effects. It is a "two-stage" procedure. Firstly, effect sizes and variances are estimated for each study and secondly, these individual estimates are combined to calculate pooled effects. When zero cell frequencies are observed in a 2 by 2 contingency table of vaccination/control by shedding/non-shedding, the inverse variance method uses a continuity correction factor (usually 0.5) in order to estimate the effect size and its variance which may bias the results. For example, when the outcome frequency was low and the number of treated and controls individuals were not balanced, Sweeting et al. (2004) observed that the inverse variance method could cause more biased results than logistic regression. When study outcomes are binary and the frequency of vaccinated and control animals developing the outcome is stated, then a "one-stage" approach using logistic or log-binomial models can be used (Sweeting et al. 2004; Simmonds and Higgins 2014). However, convergence

problems were encountered when attempting the log-binomial option with random slopes for vaccination status to model urinary shedding. This was possibly due to the frequency of zero counts for animals shedding leptospires in most vaccinated groups across the meta-analysis. Although, shedding was usually common among unvaccinated animals and most trials were balanced as they used a similar number of vaccinated and unvaccinated animals, it is likely that the 82.1% vaccine efficacy is a conservative estimate due to the use of 0.5 as a continuity correction factor.

5.6 Conclusion

Vaccination against Hardjo in cattle and deer prior to challenge reduces the risk of urinary shedding as measured by bacteriological culture. It is likely that the 70.8% - 89.0% vaccine efficacy confidence interval range estimated, when used in a long-term vaccination programme, is sufficient to achieve long-term herd immunity, decreasing the chance for human exposure, but this is yet to be determined. The mean vaccine efficacy of 82%, estimated by the meta-analysis of culture results, can be used to inform preventive strategies for reducing leptospirosis incidence in humans and evaluate the economic effect of vaccination in endemically infected herds. Although vaccine efficacy tended to be higher in trials using monovalent compared with trials using vaccines with $\geq$2 serovars, further evidence is required to confirm that this association is significant under comparable trial conditions.

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Chapter 6

General discussion

6.1 Introduction

In New Zealand, human leptospirosis has been a notifiable disease since 1952 (Christmas et al. 1974b). Back in those days, dairy cattle farmers and workers were the ones mostly affected by the disease. Although human cases were significantly reduced at the beginning of the 1980s, probably due to the use of a leptospiral vaccine in dairy cattle and leptospirosis awareness campaigns (Marshall 1987; Marshall and Manktelow 2002), the annual rate of human notifications is still one of the highest among high income countries (Pappas et al. 2008).

Leptospirosis continues to be an occupational disease since it affects mostly people working in close contact with livestock species. Surveillance data from 2001 to 2014 showed that on average 80% of notified cases were either abattoir workers or farmers (ESR 2002-2015). Recent investigations aimed to quantify the level of *Leptospira* sero-positivity in abattoir workers. Benschop et al. (2009) observed that 5.4% (n=242) of workers of a predominately sheep abattoir, were sero-positive (MAT $\geq$24) for Pomona and 4.1% (n=242) for Hardjo-bovis. In another serological survey of meat workers in cattle, sheep or deer abattoirs, Dreyfus et al. (2014) observed a sero-prevalence of 8% to Hardjo-bovis and 5% to Pomona using a higher titre cut-off (MAT $\geq$48). No other serovars were tested in these studies, because they were not deemed to play a major role for infection in abattoir workers. In the Waikato region of New Zealand, Cowie and Bell (2012) observed that among 17 notified cases working in meat processing industry, 10 were serologically positive for Pomona and five for Hardjo-bovis. One person was sero-positive for Tarassovi (5.9%) and none for Ballum. This may suggest that the majority of leptospirosis cases among abattoir workers were caused by Pomona and Hardjo-bovis, and very few by Ballum, Tarassovi or Copenhageni. However, no estimates are available to date on sero-positivity or illness attributed to the latter serovars, neither for abattoir workers nor for other occupations at risk of leptospirosis.

6.2 Under-ascertainment of human leptospirosis

Human leptospirosis causes a variety of signs and symptoms that may resemble influenza or other febrile illnesses, such as dengue or malaria in tropical climates. Commonly, people suffering from severe leptospirosis were observed to develop fever,

myalgia, headache, nausea or vomiting and photophobia (Christmas et al. 1974a; Vickery et al. 2006). This is considered to be one contributing factor to under-ascertainment of leptospirosis worldwide. Moreover, in low income countries there may be a comparatively lower access to health care and scarcity of resources for disease diagnosis. In a collaborative effort to estimate the global morbidity and mortality of leptospirosis, Costa et al. (2015) estimated that at least 1.03 million leptospirosis cases occur in the world every year. Nevertheless, this estimate is likely to represent only severe cases of disease that also were more likely to seek medical attention, and be diagnosed.

In New Zealand, despite relatively good access to health care and diagnostic laboratory services, human leptospirosis is likely to be under-ascertained, particularly for mild clinical cases of disease. In addition to the severity of clinical signs, the need to demonstrate a 4-fold rise in antibody titres in paired samples or isolation of *Leptospira*, required for compensation by Accident Compensation Corporation (ACC), contributed to under-ascertainment of disease. Recently, cases may be confirmed by detection of leptospiral DNA from a clinical sample using PCR (Mansell and Benschop 2014). This may improve the detection of cases and reduce under-ascertainment in the future. A general lack of awareness may also have an impact on notifications. The awareness of medical staff or general practitioners about leptospirosis has not been quantified but it may be assumed to be high, at least when people working in occupations at risk of exposure show signs and symptoms compatible with leptospirosis. On the other hand, awareness of people working in occupations at risk of leptospirosis may vary according to the occupation. It is possibly that in the meat industry there is a comparatively higher awareness than in farmers of beef cattle, sheep or deer. Anecdotally, during the time of contacting people for participation in the study on *Leptospira* sero-positivity in farmers of beef cattle, sheep and deer (Chapter 2), some farmers were surprised that leptospirosis could affect them since it was considered "a disease of dairy cattle" or "that affected cattle only", not sheep or deer. In the light of these reasons, the annual average of 2.24 cases per 100,000 people notified in New Zealand during the last 14 years (ESR 2002-2015) is likely to represent the severe end of the spectrum of clinical signs and symptoms that people exposed to *Leptospira* may experience.

To quantify the rate of under-ascertainment of leptospirosis in New Zealand, the level of *Leptospira* sero-positivity in at risk populations had to be estimated.

Furthermore, the association between serology and illness needed to be assessed in order to make inferences on the expected annual number of leptospirosis cases.

6.3 *Leptospira* sero-positivity and risk factors in farmers and veterinarians

In addition to abattoir workers, farmers and farm workers are occupational groups that are regularly included in the notified case statistics (ESR 2002-2015). We decided to target farmers of beef cattle, sheep, and/or deer for inclusion in our serological survey since these species, in contrast to dairy cattle or pigs, are rarely vaccinated against leptospirosis. Also, previous serological surveys of beef cattle, sheep, and deer observed that they were highly sero-positive to Hardjo-bovis and Pomona (Ayanegui-Alcerreca et al. 2010; Dreyfus et al. 2011). Since livestock serology results across New Zealand were available from the survey of farms conducted by Dreyfus et al. (2011), these data represented an opportunity for assessing the level of *Leptospira* sero-positivity of farmers working on those farms. Veterinarians on the other hand, have not been common among notified cases in recent years, despite their close contact and frequent exposure to livestock species. Nevertheless, since the population of veterinarians in New Zealand is about 2,000 people, they are not expected to be reported in high numbers. Comparatively, there are 10-fold as many abattoir workers as veterinarians in New Zealand. Thus, if the intensity of exposure were the same, one notified leptospirosis case in veterinarians would be equivalent to 10 leptospirosis cases in abattoir workers. Previous serological surveys in veterinarians have demonstrated sero-positivity to *Leptospira* (Robinson and Metcalfe 1976; Blackmore and Schollum 1983) but they were recorded at times of high national incidence and recent estimates were not available.

In the serological studies conducted in veterinarians (Chapter 3) and farmers of beef cattle, sheep, and deer (Chapter 2), the five most prevalent serovars in notified cases were tested. Results showed that Pomona was the most prevalent serovar in veterinarians (2.5%; 95% CI 1.0%–5.1%) and farmers (2.6%; 95% CI 0.9%–5.7%). However, contrasting results were observed for serovar Hardjo-bovis, which was the second most common serovar in veterinarians (2.2%; 95% CI 0.8%–4.7%) but none of the 178 farmers working in 127 farms was positive for Hardjo-bovis at the MAT titre

cut-off of ≥48. Instead, the second most prevalent serovar in farmers was Ballum (2.1%; 95% CI 0.6%–4.9%), a serovar that was commonly observed in past serological surveys of hedgehogs and rats in New Zealand (Hathaway and Blackmore 1981; Hathaway et al. 1981) and is not included in animal vaccines. These free-living species may act as reservoirs for infection in domestic livestock and humans (Brockie and Till 1977). It is therefore prudent that future research addresses *Leptospira* species in wildlife and their molecular similarity to *Leptospira* in livestock and people.

Risk factors for *Leptospira* sero-positivity were identified for veterinarians and farmers of beef cattle, sheep, and deer. Veterinarians appeared to be at higher risk of *Leptospira* sero-positivity when slaughtering cattle or pigs at home, and when exposed to multiple species at work (Mid-low and mid high dog-cat exposure), while farmers appeared to be at a higher risk of sero-positivity when assisting calving of cattle or deer, farming deer alone or mixed with beef cattle or sheep, and when the farm was on predominately flat terrain or high abundance of wild deer was reported on farm. Home slaughter was not a risk factor for *Leptospira* sero-positivity in farmers, despite that most farmers (154/178) were involved in this activity. The three percent difference in sero-prevalence between farmers slaughtering (7%) and not slaughtering animals at home (4%) was not statistically significant. Although we explored the association between sero-status and the number of animals slaughtered in a year or the species slaughtered by the farmers, we still did not observe a significant association. Similar results were observed in a serological survey in abattoir workers, where home slaughter was not a risk factor for *Leptospira* sero-positivity (Dreyfus et al. 2014). It may be possible that the additional risk of *Leptospira* infection in farmers and abattoir workers due to home slaughter is comparatively lower than the additional risk of infection in veterinarians performing this activity.

Enhancing awareness of factors associated with *Leptospira* sero-positivity may lead to implementation of interventions designed to reduce the risk of infection. This may be particularly useful for activities such as home slaughter, assisting calving, and deer farming. Also, the use of protective equipment (i.e. gloves, spectacles) may further protect people performing these identified activities. For other potential risk factors as the abundance of wild deer and flat terrain on farm, further investigations are required to better understand the role of wildlife and topography on human infection.

Leptospira sero-prevalence studies conducted in abattoir workers, farmers of beef cattle, sheep and/or deer; and veterinarians provided not only an indication of the current level of *Leptospira* sero-positivity, serovar frequencies, and risk factors associated with the serological status of participants; but also an estimate of influenza-like illness incidence due to *Leptospira* infection.

6.4 *Leptospira* sero-positivity and influenza-like illness

Dreyfus et al. (2015) conducted a prospective serological study in abattoir workers and observed an association between new Hardjo-bovis and/or Pomona infection and influenza-like illness, as defined as an episode of illness accompanied by fever, headache, myalgia, photophobia and/or nausea/vomiting. From these data, the annual incidence of illness attributed to leptospirosis was estimated. The importance of this assessment lies on that it allows estimating the total number of severe and mild infections expected to occur in a year, giving an indication of the extent of under-ascertainment of leptospirosis in abattoir workers (16–56 times). However, the incidence risk of illness due to *Leptospira* and under-ascertainment of disease for other occupational groups at risk of leptospirosis was yet unknown.

From the cross-sectional studies targeting veterinarians and farmers of beef cattle, sheep, and/or deer described in Chapters 3 and 2, respectively; *Leptospira* sero-positivity was associated with episodes of influenza-like illness episodes that occurred 18 months prior to sampling. It was observed that the annual incidence of illness (mild and severe) attributed to Hardjo-bovis, Pomona, Ballum, Tarassovi, and/or Copenhageni infection was 0.5% and 1.3% for veterinarians and farmers, respectively. The annual incidence of disease attributed to *Leptospira* infection in veterinarians and farmers was lower than the annual incidence of illness attributed to Hardjo-bovis and/or Pomona sero-conversion (2.7%) in abattoir workers.

Because a cross-sectional study design was used for estimating the sero-prevalence of *Leptospira* in veterinarians and farmers, it was not possible to determine whether infection occurred before or after an illness episode. Nevertheless, this association

was consistently observed in abattoir workers (RR=1.9), veterinarians (RR=1.4), and farmers (RR=1.8). It also is biologically plausible that the direction of causality was from *Leptospira* infection to influenza-like illness.

Alternatively, we could have opted for sampling veterinarians and farmers at least twice in a year, and record the incidence of influenza-like illness by asking participants to recall illness episodes. This would have removed residual antibody titres from past infections and strengthen the causal relationship. However, even such a design would not necessarily have demonstrated causality since some individuals could have been exposed to *Leptospira* and sero-converted after an unrelated episode of influenza-like illness, or infected and not develop clinical signs. Hence, close surveillance of subjects and regular blood samplings would have been required to reduce the possibility of a spurious association. Feasibility of such a design would have been compromised by a lower participation rate, lost to follow-up of participants, demanding logistics, and limited resources. Nevertheless, due to repeatedly observing significant associations between *Leptospira* sero-positivity and influenza-like illness, we became increasingly confident that a proportion of influenza-like cases in the study populations were truly attributable to *Leptospira* infection.

6.5 Burden of leptospirosis in New Zealand

An important metric for quantifying the burden of a disease is the Disability-Adjusted Life Years (DALYs), which indicates the number of years lost due to illness, disability or death (WHO 2016). Recent WHO initiatives encouraged leptospirosis global burden assessment (WHO 2011). Two recent reviews provided worldwide estimates of morbidity, mortality, and DALYs (Costa et al. 2015; Torgerson et al. 2015). Nevertheless, the impact of less severe forms of leptospirosis was largely ignored due to the lack of incidence data. Our estimated incidence of influenza-like illness attributed to *Leptospira* infection (PAR) in veterinarians (Chapter 3) and farmers (Chapter 2), together with the previous estimation in abattoir workers (Dreyfus et al. 2015), allowed estimating the annual number of mild and severe human leptospirosis cases in people working in at risk occupations in New Zealand. Consequently, we estimated in a stochastic simulation the annual DALYs due to mild, severe, and chronic leptospirosis in New Zealand by combining the expected number of incident cases, information on disease duration (Goris et al. 2013; Drey-

fus et al. 2015), and assumptions on disability due to illness (Salomon et al. 2012). Moreover, while human leptospirosis was believed to be under-ascertained, an estimate of the extent of under-ascertainment in the country is now available for the first time. These results contribute not only towards quantifying the global burden of leptospirosis, but also inform public health and surveillance agencies in New Zealand. Possible benefits may arise for the future allocation of resources for health control and for revising surveillance strategies.

To estimate the monetary cost of leptospirosis in New Zealand, values of hospitalisation, treatment, absence from work due to illness, production loss in livestock, and animal vaccination to prevent disease were retrieved, and included in a stochastic simulation. Results from several recent studies of livestock productivity were available for this analysis (Ayanegui-Alcerreca 2006; Subharat et al. 2011; Subharat et al. 2012a; Sanhueza et al. 2013; Vallée et al. 2016b; Vallée et al. 2016a). The estimated annual cost of leptospirosis was NZ$25.36 million, half of which was for vaccinating dairy cattle (which represented 92% of the total cost of livestock vaccine). Human illness constituted 18% of the total cost of leptospirosis. This may raise the question whether vaccination is cost-effective, a difficult one to answer since the real effect on prevention of human cases would be revealed only if annual vaccination of dairy cattle were not performed.

Recently, a cluster of three workers with leptospirosis occurred on a dairy farm. Although vaccination was performed, insufficiencies in the way the vaccine was used were observed but not detailed (McLean et al. 2014). Furthermore, the investigation revealed that 16 animals of unknown vaccination status were incorporated to the herd a month before the outbreak. Also, leptospirosis outbreaks in dairy workers of non-vaccinated farms have been anecdotally reported. This strongly suggests that in the absence of effective annual vaccination, the number of human cases and associated cost would be substantially higher than currently observed in New Zealand or estimated by our study.

6.6 Control of leptospirosis

All *Leptospira* vaccines registered in New Zealand include Hardjo and Pomona antigens. Additionally, two vaccines provide protection against Copenhageni. Vac-

cination is likely to be the most effective way of preventing leptospirosis in animals and reducing *Leptospira* infection in humans (Marshall 1987). Although most ($\sim$ 90%) dairy cattle are annually vaccinated against leptospirosis, its use is still limited in beef cattle, sheep, and deer farms.

Several vaccination trials have assessed vaccine efficacy to prevent shedding of leptospires in urine of cattle. Nevertheless, no mean estimate of vaccine efficacy was available. Moreover, factors influencing the performance of leptospiral vaccines had not been formally assessed. Therefore, we conducted a systematic review of studies evaluating the protection conferred by commercial Hardjo and/or Pomona vaccines against urinary shedding of leptospires after natural or artificial conjunctival challenge of cattle, sheep, and deer. Individual trials gathered in the systematic search were included in a meta-analysis that resulted in a pooled mean vaccine efficacy of 82.1% when bacteriological culture was used to detect leptospires in urine (Chapter 5). It is possible that the estimated 82.1% vaccine efficacy is above the threshold to stop extensive transmission and trigger herd immunity. Nonetheless, this estimate represents the average protection against shedding in trials conducted under experimental conditions. The level of natural or artificial *Leptospira* exposure in experimental trial conditions was likely to be higher than the expected when all animals in a herd are annually vaccinated. Therefore, the efficacy of a vaccination programme is likely to be higher than the individual vaccine efficacy reported in Chapter 5. On the other hand, trials resulting in vaccine failure may be less likely to be reported, which would render the estimate from the meta-analysis of this thesis as optimistic. More importantly though, as *Leptospira* vaccines are certainly not 100% efficacious, their effectiveness at herd level depends on infection pressure, contact rate, host and environmental factors, which differ greatly between farms and populations. An 82% efficacious vaccine may well be suited for achieving "herd immunity" (i.e. a breakdown of transmission leading to absence of infection), but demonstrating this in the field would require substantial resources. In this instance, mathematical models may be used for determining the critical threshold for vaccine efficacy to achieve herd immunity.

Vaccination should reduce the risk of *Leptospira* infection in animals and humans working in close contact with them. However, it only prevents against the serovars that are included in the vaccine since surface antigens of *Leptospira* from different serogroups do not cross-protect. Although Hardjo-bovis and Pomona were

the two most common serovars in human notified cases from 2001 to 2014, Ballum infections have increased in recent years being the second most commonly observed after Hardjo-bovis since 2008 (ESR 2002-2015). Ballum was typically associated with free-living species (e.g. hedgehogs), but sero-positivity of sheep and deer to this serovar has also been reported (Blackmore et al. 1982; Wilson et al. 1998). The source of Ballum infection in human cases remains unclear and should be further investigated.

The main factor limiting the widespread adoption of vaccination from the farmer's point of view is cost-benefit since production loss attributed to leptospirosis are in most cases subtle, at least in beef cattle and sheep (Vallée et al. 2014). Although farmed deer in New Zealand are maintenance host for Hardjo-bovis (Subharat et al. 2012b), it seems that they are comparatively less adapted to this serovar compared to cattle or sheep. Production loss in deer, potentially attributed to *Leptospira* infection, were observed in two vaccination trials where unvaccinated deer showed a reduced weaning rate (Ayanegui-Alcerreca 2006; Subharat et al. 2011) and lower average daily gain compared with vaccinated deer (Subharat et al. 2012a), or where a reduced growth rate was observed in unvaccinated deer without evidence of *Leptospira* infection compared with unvaccinated deer naturally infected (Ayanegui-Alcerreca 2006). In Chapter 4 we estimated the monetary value associated with production loss due to leptospirosis in beef cattle, sheep, and deer. This resulted in a median annual production loss estimate of NZ$12.5 per hind, which was higher than the median production loss estimated for beef cows (NZ$2.0) and ewes (NZ$0.1), respectively. This suggested that the adoption of a vaccination programme in deer may have a comparatively higher monetary return than in beef cattle and sheep by reducing production loss possibly associated with leptospirosis. Furthermore, in Chapter 2 we observed that farming deer, as opposed to not farming deer, was a risk factor for *Leptospira* sero-positivity of farmers. Also, a serological survey in abattoir workers of deer plants observed that 10/57 (17.5%) were sero-positive to Hardjo-bovis and/or Pomona compared to 10/185 (5.4%) and 42/325 (12.9%) in sheep and beef plants, respectively (Dreyfus et al. 2014). In addition to production gains, vaccination of deer may therefore benefit the health of deer farmers directly, and indirectly abattoir workers of deer plants, by reducing the occurrence of mild and severe illness. A vaccination programme on deer farms would benefit the public health sector and meat industry by reducing the number of people hospitalised, treated and not able to work due to leptospirosis. The cost benefit of *Leptospira*

vaccination in deer should be further assessed in the country without ignoring the potential benefits outside farm boundaries.

6.7 Methodology critiques

6.7.1 Microscopic agglutination test

The microscopic agglutination test (MAT) was used to detect antibodies against *Leptospira*. It is considered a highly specific test in convalescent samples but its sensitivity and specificity can be low during the early acute phase of the disease (Levett 2001). A Bayesian latent class analysis showed that sensitivity and specificity of MAT alone, for diagnosis of acute samples (MAT$\geq$400 or 4-fold rise in MAT titre), were 49.8% and 98.8%, respectively (Limmathurotsakul et al. 2012). However, in the sero-prevalence studies conducted in veterinarians (Chapter 3) and farmers (Chapter 2) a lower MAT titre cut-off of $\geq$48 for a positive sample was used. This was done to assess past infection to *Leptospira* in healthy veterinarians and farmers, not clinical illness. By reducing the MAT titre cut-off, sensitivity of the test may have been improved to some extent but a decrease in specificity could have occurred concurrently. The MAT is a serogroup specific test (Levett 2001), and therefore cross-reactions can occur between serovars from the same serogroup. In New Zealand however, few serovars are endemic; and in human infections, only five serovars (Hardjo-bovis, Pomona, Ballum, Tarassovi and Copenhageni) have been observed regularly in notified cases. Thus, the chance of cross-reaction between these serovars that belong to five different serogroups is believed to be limited. Although sensitivity and specificity of the MAT using the cut-off of $\geq$48 are not perfect, no adjustment was done for *Leptospira* sero-prevalence estimates of veterinarians and farmers since no data were available on the accuracy of MAT using this titre cut-off in human samples.

6.7.2 Antibody titre duration

Duration of antibody titres in humans is highly variable but there is a general consensus that they can last for several years after infection (Blackmore et al. 1984; Levett 2001). Although MAT titres reflect past infection to *Leptospira*, they do not

always correlate with protection against challenge, at least for serovar Hardjo-bovis (Bolin et al. 1989; Bolin et al. 1991; Zuerner et al. 2011). The potential continuous re-exposure and re-infection of individuals imposes a challenge to determine antibody titre duration after a single infection. In the serological studies conducted in abattoir workers (odds ratio=10.3), veterinarians (prevalence ratio=8.9), and farmers (prevalence ratio=8.5); people that recalled being diagnosed with leptospirosis at some point in their lives were strongly associated with an existing MAT titre $\geq$48 (sero-positive for analysis purposes). Most (75%) of past leptospirosis episodes in veterinarians and farmers occurred from 9 years to 40 years prior to serology assessment, suggesting that people suffered from leptospirosis in the past were more likely to be re-infected. In terms of antibody titre duration in humans, Dreyfus et al. (2015) estimated that Hardjo-bovis and Pomona MAT titres $\geq$48 were maintained on average for 29 and 10 months, respectively. Therefore, sero-prevalence studies can offer insights about past *Leptospira* infection in the population being studied.

6.7.3 Recalling influenza-like illness

We relied on participant veterinarians and farmers to remember episodes of influenza like-illness, defined as an episode of illness associated with fever, myalgia, headaches, sweating, and severe general debility in the 18 months prior to participation in the study. This time frame was chosen to cover most of the average titre duration after *Leptospira* infection while compromising as little as possible the recalling accuracy of disease episodes. One may speculate that the ability of an average person to recall an influenza-like illness episode beyond 12 months is comparatively lower than in the first year and may decrease with the experienced illness severity. By scaling the observed population attributable risk (PAR) of veterinarians and farmers to 12 months, instead of 18 months, we were able to associate influenza-like illness incidence with *Leptospira* infection in a year. However, the question that remained was what would have happened if we asked to recall illness in the last 12 months only? If the answer to this question is that we would have observed a similar association, then disease incidence estimates were unnecessarily reduced, underestimating the annual expected number of leptospirosis cases, burden, and associated cost of disease. Hence, our estimates of population impact would have been underestimated too by approximately 24% for DALYs and 5% for total economic cost.

6.8 Future research

6.8.1 Leptospirosis in dairy cattle

Most dairy herds in New Zealand vaccinate their animals annually against leptospirosis. However, a pilot study revealed that 30% of herds (13/44), which had been vaccinated for more than five consecutive years, and 13% of animals from positive herds (18/134), were dark field microscopy (DFM) or PCR positive to *Leptospira* in urine (Wilson et al. 2013). It was also observed that herds vaccinating <3 months old calves were less likely to have cows shedding *Leptospira* in urine than herds starting to vaccinate calves later. This raised the question of whether bacteria being shed in urine belonged to serovars contained in commercial vaccines. If so, it may be possible that infection was already occurring in older animals at the time of vaccination. Under this scenario, an important consideration is the duration of maternal derived antibodies (MDA) in new-born animals and the potential interference between MDA and vaccination, if animals were to be vaccinated at a young age against leptospirosis. In Chapter 1, we reviewed the use of livestock vaccination as a means for controlling leptospirosis. We found no evidence in the literature to support this presumed interference between MDA and vaccination but only few studies have tested this effect. Another question is whether PCR-positive urine is infectious for in-contact humans. PCR is regarded as a more sensitive method than culture but it detects DNA from both viable and dead bacteria. The epidemiological importance of a PCR positive result should be evaluated quantitatively, as vaccinated animals may shed leptospires in numbers below infectious threshold. A third consideration in this study is that PCR-positive cows were infected with serovars not included in the vaccine.

A PhD project is currently underway aiming to describe vaccination practices in New Zealand dairy herds, to assess the prevalence of urinary shedding, and to determine the serovar(s) associated with shedding. Results are expected to contribute towards the understanding of the efficiency of current vaccination in dairy herds.

6.8.2 Leptospirosis in wildlife

Serological surveys conducted during the 1970-1980s in New Zealand, established that several free-living species as rats, hedgehogs, feral goats and deer, had serologi-

cal evidence of some *Leptospira* serovars (Hathaway and Blackmore 1981; Hathaway et al. 1981; Schollum and Blackmore 1981; Inglis 1984). Hedgehogs were suggested to be a reservoir for Ballum infection in dairy cattle (Brockie and Till 1977). Nevertheless, the sero-prevalence to *Leptospira* in wildlife species has not been assessed in recent years. Furthermore, the direct or indirect role that wildlife species have on farmed animals, vaccinated dairy herds, and human infection remains unknown. A PhD project addressing these issues is expected to start in 2016.

6.8.3 Sero-prevalence to serovars other than Hardjo-bovis and Pomona in livestock

Although sero-positivity to Ballum and Copenhageni was observed in farmed cattle, sheep and deer (Ris et al. 1973; Blackmore et al. 1982; Flint et al. 1988), there are no current estimates of the level of sero-positivity to Ballum, Tarassovi, and Copenhageni in these species. These serovars are frequently observed in human notified cases; nonetheless, the potential role of domestic livestock on human infection for these serovars has not been assessed. The task of testing stored sera samples of beef cattle, sheep, and deer for antibodies against Ballum, Tarassovi, and Copenhageni using the MAT has been completed recently and revealed a number of findings. A report is expected by end of 2016.

6.8.4 Spatial analysis of animal's serology data

Using the spatial point location of farms and the complete set of serology data against Hardjo-bovis, Pomona, Ballum, Tarassovi, and Copenhageni in beef cattle, sheep, and deer, density risk maps can be constructed to identify areas of high or low sero-prevalence in the country. This can be particularly useful for serovars not usually associated with livestock infection since any high risk areas can be targeted for further investigation on *Leptospira* exposure in domestic animals, wildlife, and humans. In addition, available spatial data include soil characteristics, which may well be linked to pathogen survival.

6.8.5 Post-acute leptospirosis sequelae

Human leptospirosis can lead to long lasting sequelae, which can affect the ability of affected people to re-establish full health and regain the same quality of living and working ability they had prior to illness. In The Netherlands, post-acute sequelae were observed to occur in 30.2% of people that suffered from leptospirosis (Goris et al. 2013). The extent of this problem has not been assessed in New Zealand. Further research should aim to describe the occurrence, nature, and effects of such post-acute leptospirosis sequelae.

6.8.6 Serological surveys in other at risk occupations

Serological studies in occupational groups potentially at risk of leptospirosis such as stock truck drivers, shearers, butchers, food processors, and horse farm workers, only to mention some, have not been done. Because of the widespread use of vaccination against leptospirosis in most dairy and pig herds, low exposure, at least to Hardjo-bovis and Pomona, may be expected in dairy and pig farm workers. However, serological surveys of humans may useful for detecting further associations with vaccination of animals and likely infection sources. Moreover, the relative importance of other *Leptospira* serovars, not currently present in registered vaccines for use in cattle or pigs, for human disease could be uncovered.

6.8.7 Analysis of human notified cases

Another area that can be further explored is the epidemiological investigation of notified cases. Although a description of notified cases is presented annually by The Institute of Environmental Science and Research Ltd. (ESR), surveillance data should be analysed from time to time. For example, it would be of interest to stratify leptospirosis farmer cases by species they work with in more detail than currently included in annual surveillance reports. Moreover, frequency comparisons of serovars reported in each farming category could be formally tested. Since most dairy herds vaccinate against leptospirosis, it is of relevance to know the proportion of notified dairy workers that potentially contracted the disease in dairy farms where animals were regularly vaccinated. Furthermore, infecting serovars from individuals of vaccinated farms could be compared with the specific serovar frequencies observed

in notified cases from unvaccinated farms. Finally, for those vaccinated farms where either Hardjo-bovis or Pomona was identified as the infecting serovar in human notified cases, potential reasons for vaccination programme failure could be described in order to strengthen prevention of leptospirosis in animals and humans.

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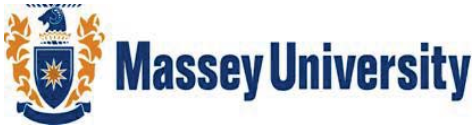
Appendices

.1 Appendix I: Farmer questionnaire



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Study of Leptospirosis in Farm Personnel

Participant Questionnaire

The research team appreciates your involvement in this study of leptospirosis.

The information from this questionnaire will help us to assess the risk of contracting leptospirosis on farms and to develop control strategies.

This study and questionnaire have been approved by the Massey University Human Ethics committee.

Personal information included in the questionnaire will be treated in confidence and will not be published or disclosed to any third parties (for example your employer) by the research team in a manner that would allow identification of participants.

Results will be communicated to you either by mail or email.

Date of interview: ____/____/2013 Interviewer's name: _____

1. Participant general information

Name & Surname	
Did you fill in a consent and confidentiality form?	☐ Yes ☐ No (Please get both forms filled in now)
Farm name and physical address	
Your postal address (if different)	
Email	
Communication of results	☐ Mail ☐ Email
Contact phone number	
Date of Birth	_____/_____/_____ (day/month/year)
Gender	☐ Female ☐ Male
With which ethnic affiliation do you identify with?	☐ NZ-Maori ☐ Pacific Islander ☐ NZ-European ☐ Asian ☐ Other_____
How long have you been working on **this** farm?	_____ years
For how long have you been working in livestock farming?	_____ years
Do you smoke on the job or during breaks at work?	☐ Yes ☐ No
Do you suffer from hay fever?	☐ Yes ☐ No

2. Exposure at primary workplace:

2.1 Quantify your **exposure** (direct physical contact) to different animal species during the **last 18 months** (e.g. during lambing, calving, tailing, weaning, drenching, etc.). Please indicate **how many animals on average** you were in contact with in different seasons of the year and state the frequency of this contact at the scale: **0 (no contact)** to **10 (everyday contact).**

Type	Animal Numbers	Frequency of contact (please circle a number)	Leptospira vaccination
Dairy cattle	Dec-Feb'12 _____	0 1 2 3 4 5 6 7 8 9 10	☐ Yes (date: mm/yy) _____
	Mar-May'12 _____	0 1 2 3 4 5 6 7 8 9 10	
	Jun-Aug'12 _____	0 1 2 3 4 5 6 7 8 9 10	☐ No
	Sep-Nov'12 _____	0 1 2 3 4 5 6 7 8 9 10	
	Dec-Feb'13 _____	0 1 2 3 4 5 6 7 8 9 10	☐ Don't know
	Mar-May'13 _____	0 1 2 3 4 5 6 7 8 9 10	
Beef cattle	Dec-Feb'12 _____	0 1 2 3 4 5 6 7 8 9 10	☐ Yes (date: mm/yy) _____
	Mar-May'12 _____	0 1 2 3 4 5 6 7 8 9 10	
	Jun-Aug'12 _____	0 1 2 3 4 5 6 7 8 9 10	☐ No
	Sep-Nov'12 _____	0 1 2 3 4 5 6 7 8 9 10	
	Dec-Feb'13 _____	0 1 2 3 4 5 6 7 8 9 10	☐ Don't know
	Mar-May'13 _____	0 1 2 3 4 5 6 7 8 9 10	
Sheep	Dec-Feb'12 _____	0 1 2 3 4 5 6 7 8 9 10	☐ Yes (date: mm/yy) _____
	Mar-May'12 _____	0 1 2 3 4 5 6 7 8 9 10	
	Jun-Aug'12 _____	0 1 2 3 4 5 6 7 8 9 10	☐ No
	Sep-Nov'12 _____	0 1 2 3 4 5 6 7 8 9 10	
	Dec-Feb'13 _____	0 1 2 3 4 5 6 7 8 9 10	☐ Don't know
	Mar-May'13 _____	0 1 2 3 4 5 6 7 8 9 10	
Deer	Dec-Feb'12 _____	0 1 2 3 4 5 6 7 8 9 10	☐ Yes (date: mm/yy) _____
	Mar-May'12 _____	0 1 2 3 4 5 6 7 8 9 10	
	Jun-Aug'12 _____	0 1 2 3 4 5 6 7 8 9 10	☐ No
	Sep-Nov'12 _____	0 1 2 3 4 5 6 7 8 9 10	
	Dec-Feb'13 _____	0 1 2 3 4 5 6 7 8 9 10	☐ Don't know
	Mar-May'13 _____	0 1 2 3 4 5 6 7 8 9 10	
Pig	Dec-Feb'12 _____	0 1 2 3 4 5 6 7 8 9 10	☐ Yes (date: mm/yy) _____
	Mar-May'12 _____	0 1 2 3 4 5 6 7 8 9 10	
	Jun-Aug'12 _____	0 1 2 3 4 5 6 7 8 9 10	☐ No
	Sep-Nov'12 _____	0 1 2 3 4 5 6 7 8 9 10	
	Dec-Feb'13 _____	0 1 2 3 4 5 6 7 8 9 10	☐ Don't know
	Mar-May'13 _____	0 1 2 3 4 5 6 7 8 9 10	

Goat	Dec-Feb'12 _____	0 1 2 3 4 5 6 7 8 9 10	☐ Yes (date: mm/yy) _____
	Mar-May'12 _____	0 1 2 3 4 5 6 7 8 9 10	☐ No
	Jun-Aug'12 _____	0 1 2 3 4 5 6 7 8 9 10	
	Sep-Nov'12 _____	0 1 2 3 4 5 6 7 8 9 10	☐ Don't know
	Dec-Feb'13 _____	0 1 2 3 4 5 6 7 8 9 10	
	Mar-May'13 _____	0 1 2 3 4 5 6 7 8 9 10	
Other (Specify)	Dec-Feb'12 _____	0 1 2 3 4 5 6 7 8 9 10	☐ Yes (date: mm/yy) _____
	Mar-May'12 _____	0 1 2 3 4 5 6 7 8 9 10	☐ No
	Jun-Aug'12 _____	0 1 2 3 4 5 6 7 8 9 10	
	Sep-Nov'12 _____	0 1 2 3 4 5 6 7 8 9 10	☐ Don't know
	Dec-Feb'13 _____	0 1 2 3 4 5 6 7 8 9 10	
	Mar-May'13 _____	0 1 2 3 4 5 6 7 8 9 10	

2.2 Exposure to Animal Urine and cleaning

In your opinion, what activity in your farm work puts you more at risk of animal urine exposure?	
Over the last 18 months, were you working in close physical contact with animals, such that animal urine might have come in direct contact with skin, nose, eyes, mouth?	☐ Yes ☐ No, **Go to section 2.3**
If yes, did you wash or clean hands after work (tick only one)?	☐ Regularly ☐ Sometimes ☐ Rarely ☐ Never
If you washed or cleaned hands, what did you use (tick what applies)?	☐ Tap water ☐ Water from trough ☐ Water from river, pond or lake ☐ Paper towel only ☐ Fabric towel only ☐ Other_____

2.3 Exposure to Animal Urine

<u>In the last 18 months</u> have you been involved in any of the following activities?

Activity	**Involved in the last 18 months?**	**Protective measures <u>routinely</u> used (tick as appropriate)**
Assisting lambing	☐ Yes Average number of animals:_____ ☐ No	☐ None ☐ Use of gloves ☐ Washing hands right after activity ☐ Use of disinfectant right after activity ☐ Other (specify)_____

Assisting calving or fawning	☐ Yes Average number of animals:_____ ☐ No	☐ None ☐ Use of gloves ☐ Washing hands right after activity ☐ Use of disinfectant right after activity ☐ Other (specify)_____
Crutching/dagging/ lambs or sheep	☐ Yes Average number of animals:_____ ☐ No	☐ None ☐ Use of gloves ☐ Washing hands right after activity ☐ Use of disinfectant right after activity ☐ Other (specify)_____
Shearing sheep	☐ Yes Average number of animals:_____ ☐ No	☐ None ☐ Use of gloves ☐ Washing hands right after activity ☐ Use of disinfectant right after activity ☐ Other (specify)_____
Cleaning urine or faeces from yard surfaces	☐ Yes Average number of times/month_____ ☐ No	☐ None ☐ Use of gloves ☐ Washing hands right after activity ☐ Use of disinfectant right after activity ☐ Other (specify)_____
Milking cows	☐ Yes Average milkings/week_____ ☐ No	☐ None ☐ Use of gloves ☐ Washing hands right after activity ☐ Use of disinfectant right after activity ☐ Other (specify)_____
Docking/ Castrating lambs or calves	☐ Yes Average number of animals:_____ ☐ No	☐ None ☐ Use of gloves ☐ Washing hands right after activity ☐ Use of disinfectant right after activity ☐ Other (specify)_____
Drenching sheep or cattle	☐ Yes Average number of animals:_____ ☐ No	☐ None ☐ Use of gloves ☐ Washing hands right after activity ☐ Use of disinfectant right after activity ☐ Other (specify)_____

3. Exposure through pets and working dogs during the last 18 months.

		Number	Regularly vaccinated against leptospira? (*including any off-label administration of livestock vaccines*)
Do you have pet-dogs in/around your house?	☐ Yes ☐ No		☐ Yes Last time (mm/yy)_____ ☐ No ☐ Don't know
Do you have working dogs?	☐ Yes ☐ No		☐ Yes Last time (mm/yy)_____ ☐ No ☐ Don't know
Do you have Cats in/around your house?	☐ Yes ☐ No		☐ Yes Last time (mm/yy)_____ ☐ No ☐ Don't know
Do you have other pets in/around your house?	☐ Yes ☐ No		☐ Yes Last time (mm/yy)_____ ☐ No ☐ Don't know

4. Non-primary work related, occasional contact with livestock

Regular contact with live animals at another farm, e.g. friend's or family's house
Over the last 18 months, have you had regular (daily or weekly) contact with animals outside your livestock farming work (e.g. friend's or family's house)?

NO ☐ Skip to **Section 5.** ☐ YES Complete table below

Animal type	**No of animals**	**Animals vaccinated against Leptospirosis?**
Beef cattle		☐ Yes ☐ No ☐ Don't know
Dairy cattle		☐ Yes ☐ No ☐ Don't know
Sheep		☐ Yes ☐ No ☐ Don't know
Goats		☐ Yes ☐ No ☐ Don't know
Deer		☐ Yes ☐ No ☐ Don't know
Pigs		☐ Yes ☐ No ☐ Don't know
Working dogs		☐ Yes ☐ No ☐ Don't know
Dogs		☐ Yes ☐ No ☐ Don't know
Cats		☐ Yes ☐ No ☐ Don't know
Other_____		☐ Yes ☐ No ☐ Don't know

5. Other regular work history*:*

Over the last 18 months, have you had any other regular work besides your farm work?

NO ☐ Skip to **Section 6.** ☐ YES Complete table below

Type of work	**Average hours per week?**	**Approximately for how long have you done this (years)?**	**When did you last do this work?**
Forestry			
Abattoir			
	Abattoir species (tick): ☐ Beef cattle ☐ Dairy cattle ☐ Sheep ☐ Goats ☐ Deer ☐ Pigs ☐ NA		
Horticulture/ cropping/ orchard			
Other (specify) _____			

6. Home slaughter (includes both that for human consumption and for dog tucker)
Do you slaughter on the farm or have you helped with home slaughtering animals elsewhere **in the past 18 months**?

☐ NO Skip to **Section 7.** ☐ YES Complete table below

Animal type	**How many per year?**	**How often per year?**	**When was the last time?**
Beef Cattle			
Dairy Cattle			
Sheep			
Goats			
Deer			
Pigs			
Other			

7. **Hunting: In the last 18 months**, have you hunted animals?

☐ NO Skip to **Section 8.** ☐ YES Please tick below

If YES, have you handled and/or slaughtered any of the animal species below (please tick)?

☐ Wild deer ☐ Wild pig ☐ Wild goats ☐ Small game*****

*****e.g. ducks, other birds, possums, rabbits, hares

☐ Other_____

8. **Outdoor exposures: Over the last 18 months**, have you been involved with outdoor activities where you were exposed to fresh water?

☐ NO **Skip to Section 9.** ☐ YES Please indicate activity:

☐ Camping beside lakes/rivers ☐ Water sports in lakes/rivers ☐ Fresh water fishing

☐ Tramping ☐ Other_____

9. **Previous illness:** Are you aware of leptospirosis and its consequences on people?

☐ Yes ☐ No

Have you ever been diagnosed with Leptospirosis?

☐ YES Complete **Section 9.1 and 9.2**

☐ NO Skip to **Section 10**

9.1 If you were ill with leptospirosis any time in the past, please enter details in the table below:

How many times did you have an illness diagnosed as leptospirosis?		
Do you know the serovar(s) (type) and/or titre(s) (blood immunity level)? Please include information from all tests if tested multiple times, starting with the most recent	Serovar(s)_____ Titre(s):_____	☐ NO

9.2 Please enter details about your last episode of leptospirosis in the table below:

Approximate month/year that you were diagnosed with leptospirosis:	_____(month/year)	
How was it diagnosed (test)?	☐ Self-diagnosed ☐ GP ☐ Blood test	
Do you know the serovar and/or titre?	Serovar: _____ Titre:	☐ No
Do you know or suspect the source of infection?	☐ Yes (specify)_____	☐ No
How many days were you off-work or seriously ill?	_____days	
Were you hospitalised?	☐ Yes (days)_____	☐ No
Which of the following flu-like symptoms did you experience?	☐ Fever ☐ Headache ☐ Sore muscles ☐ Sore eyes ☐ Sweating ☐ Severe debility ☐ Photophobia ☐ Other:_____	
Was it treated?	☐ Yes ☐ No ☐ Don't remember	
Do you think that treatment was effective?	☐ Yes ☐ No ☐ Don't know	

Do you still feel any signs of the disease or consequences now?	☐ Yes ☐ No ☐ Not sure
Received ACC compensation?	☐ Yes ☐ No ☐ Don't remember
Do you agree that we contact your GP for more information about the diagnosis and treatment?	☐ No ☐ Yes Signature:_____

10 Flu like symptoms* in the last 18 months

**e.g. fever, headache, sore muscles or bones, sore eyes, sweating, severe general debility*

Have you been ill with any flu-like symptoms in the last 18 months	☐ Yes Approx date _____	No ☐ **Skip to section 11**
In general if you have flu-like illness, would you consult a doctor?	☐ Always ☐ Mostly ☐ Rarely ☐ Never	
Have you been off work due to flu-like illness?	☐ Yes # days_____	☐ No
Were any blood tests done or samples collected?	☐ Yes ☐ No ☐ Do not remember	
Was a diagnosis made?	☐ Yes Diagnosis:_____	☐ No ☐ Do not remember
Which of the following signs of illness did you have?	☐ Fever ☐ Headache ☐ Sore muscles ☐ Sore eyes ☐ Sweating ☐ Severe debility ☐ Photophobia ☐ Other:_____	
Were you treated?	☐ Yes ☐ No ☐ Don't know/remember	
If treated, what kind of treatment did you receive?	☐ Antibiotic treatment, how many days?: _____ ☐ Other treatment, Please detail_____	

11 Your opinion

Do you believe Leptospirosis presents a serious health risk at your work?	☐ Definitely ☐ Probably ☐ Maybe ☐ No
Do you think farmers should be encouraged to vaccinate their livestock against leptospirosis? If yes, for what purpose? Please rank importance in your view, using **letter(s):** (A) To increase growth rates in animals (B) To prevent clinical disease in animals (C) To protect humans (D) To prevent reproductive loss in animals (E) To increase milk production	Dairy ☐ Yes ☐ No Letter(s)_____ Beef ☐ Yes ☐ No Letter(s)_____ Sheep ☐ Yes ☐ No Letter(s)_____ Deer ☐ Yes ☐ No Letter(s)_____ Other_____ Letter(s)_____

In your opinion, what information is required (and not available) about vaccinating animals or protecting humans against leptospirosis?	
Is there anything else you would like to comment on or say about leptospirosis in either animals or people?	

This is the end of the questionnaire. The research team appreciates your involvement in this study of leptospirosis and is committed to privacy of all personal information.

The lab will check your blood for previous exposure to Leptospira and we will notify you of the result by mail as soon as possible.

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Study of Leptospirosis in Farm Personnel

Farm Questionnaire

The research team appreciates your involvement in this study of leptospirosis.

The information from this questionnaire will help us to assess the risk of contracting leptospirosis on farms and to develop control strategies.

Name & Surname: _____

1. **In the last 4 years, has your general management of leptospirosis changed? (e.g. started vaccinating animals, stop vaccinating animals)**

☐ **No**

☐ **Yes, please explain:_____**

_____

_____

2. **Please state the average animal existences on the farm 2012 - 2013**

Type	**Animal Numbers**
Dairy cattle	
Beef cattle	
Sheep	
Deer	
Pig	
Goat	
Other (Specify)	

3. **Exposure on farm though Wildlife (rats, rabbits, possums, hedgehogs, etc.):**

Is the farm known to be prone for any of these wild animal species? Please estimate to what extent from 0=none to 5=abundant	Rabbits _____	Possums _____
	Hedgehogs _____	Rats _____
	Mice _____	Wild deer _____
	Wild pigs _____	Wild cats _____
	Stray dogs _____	Other_____

Is the farm land located next to any bush or forest?	☐ No ☐ No, but close (<500m) ☐ Small direct border ☐ Yes, next to fence
Do you use any kind of rodent control in/around the house?	☐ Yes ☐ No
Do you use any kind of rodent control in/around the place where you store animal feeds?	☐ Yes ☐ No
Are traps or poison set for possums, rabbits or hedgehogs on the farm?	☐ Yes ☐ No
Do you use gloves to handle trapped possums rabbits or hedgehogs?	☐ Yes ☐ No

4. Farming area, flooding, access to water

Please describe the farming area and soil, as percentage of land:

Flat _____% Very steep_____% Moderately steep_____% Other_____%

Clay_____% Silt_____% Sand_____%

Other_____%

5. Over the last 18 months has the farmland you work on been flooded (leaving water puddles for more than one week)?

☐ NO ☐ YES

6. Do any of the livestock species have direct access to water:

☐ No ☐ River(s) ☐ Lake(s) ☐ Valley pond(s) with dam

☐ Natural pond(s) ☐ Other:

_____

7. Water supply for humans (tick what applies):

☐ Central supply ☐ Roof water/collection tank ☐ Well ☐ Other_____

8. Water supply for animals (tick what applies):

☐ Central supply ☐ Well ☐ Valley pond with dam ☐ Natural pond

☐ River water ☐ Other_____

.2 Appendix II: Prevalence model

```
library(rjags)
mod.prev <- 'model{
    for (i in 1:num) {
y[i] ~ dbern(p[i])
logit(p[i]) <- b0
}
b0 ~ dnorm(0, 1.0E-4)
prev <- exp(b0)/(1+exp(b0))
}'

dat.log <- list(num = length(dat[,1]),
y=c(as.numeric(as.character(dat$lepto48))))
jags <- jags.model(textConnection(mod.prev),
data = dat.log, n.chains = 3, n.adapt = 5000)
results=coda.samples(jags, variable.names=c('prev'),
n.iter=30000, thin=1)
```

.3 Appendix III: Prevalence model clustering

```
library(rjags)
mod.prev <- 'model{
    for (i in 1:num) {
y[i] ~ dbern(p[i])
logit(p[i]) <- b0 + u[Herd[i]]
}
for (j in 1:m) {
    u[j] ~ dnorm(0, tau)
}
b0 ~ dnorm(0, 1.0E-4)
tau ~ dgamma(0.001, 0.001)
prev <- exp(b0)/(1+exp(b0))
}'

dat.log <- list(num = length(dat[,1]),
y=c(as.numeric(as.character(dat$lepto48))),
m=length(unique(dat$id)), Herd=c(dat$id))
jags <- jags.model(textConnection(mod.prev),
data = dat.log, n.chains = 3, n.adapt = 5000)
results=coda.samples(jags, variable.names=c('prev'),
n.iter=30000, thin=1)
```

.4 Appendix IV: Multivariable model

```
library(rjags)
mod.mult <- 'model{
    for (i in 1:num) {
y[i] ~ dbern(p[i])
logit(p[i]) <- b0 + b1*x1[i] + b2*x2[i] + b3*x3[i] + b4*x4[i] +
  b5*x5[i]
}
b0 ~ dnorm(0, 1.0E-4)
b1 ~ dnorm(0, 1.0E-4)
b2 ~ dnorm(0, 1.0E-4)
b3 ~ dnorm(0, 1.0E-4)
b4 ~ dnorm(0, 1.0E-4)
b5 ~ dnorm(0, 1.0E-4)
p1 <- 1-step(b1)
p2 <- 1-step(b2)
p3 <- 1-step(b3)
p4 <- 1-step(b4)
p5 <- step(b5)'

'rr1 <- (exp(b0 + b1*max(x1) + b2*mean(x2) + b3*mean(x3) +
b4*mean(x4) + b5*mean(x5)) / (1+exp(b0 + b1*max(x1) + b2*mean(x2)
+ b3*mean(x3) + b4*mean(x4) + b5*mean(x5)))) / (exp(b0 + b2*mean(x2)
+ b3*mean(x3) + b4*mean(x4) + b5*mean(x5))/ (1+exp(b0 + b2*mean(x2)
+ b3*mean(x3) + b4*mean(x4) + b5*mean(x5))))'

'rr2 <- (exp(b0 + b1*mean(x1) + b2*max(x2) + b3*mean(x3) +
b4*mean(x4) + b5*mean(x5))/ (1+exp(b0 + b1*mean(x1) + b2*max(x2)
+ b3*mean(x3) + b4*mean(x4) + b5*mean(x5)))) / (exp(b0 + b1*mean(x1)
+ b3*mean(x3) + b4*mean(x4) + b5*mean(x5))/ (1+exp(b0 + b1*mean(x1)
+ b3*mean(x3) + b4*mean(x4) + b5*mean(x5))))'
```

```
'rr3 <- (exp(b0 + b1*mean(x1) + b2*mean(x2) + b3*max(x3) +
b4*mean(x4) + b5*mean(x5))/ (1+exp(b0 + b1*mean(x1) + b2*mean(x2)
+ b3*max(x3) + b4*mean(x4) + b5*mean(x5)))) / (exp(b0 + b1*mean(x1)
+ b2*mean(x2) + b4*mean(x4) + b5*mean(x5))/ (1+exp(b0 + b1*mean(x1)
+ b2*mean(x2) + b4*mean(x4) + b5*mean(x5))))'

'rr4 <- (exp(b0 + b1*mean(x1) + b2*mean(x2) + b3*mean(x3) +
b4*max(x4) + b5*mean(x5)) / (1+exp(b0 + b1*mean(x1) + b2*mean(x2)
+ b3*mean(x3) + b4*max(x4) + b5*mean(x5)))) / (exp(b0 + b1*mean(x1)
+ b2*mean(x2) + b3*mean(x3) + b5*mean(x5))/ (1+exp(b0 + b1*mean(x1)
+ b2*mean(x2) + b3*mean(x3) + b5*mean(x5))))'

'rr5 <- (exp(b0 + b1*mean(x1) + b2*mean(x2) + b3*mean(x3) +
b4*mean(x4) + b5*max(x5)) / (1+exp(b0 + b1*mean(x1) + b2*mean(x2)
+ b3*mean(x3) + b4*mean(x4) + b5*max(x5)))) / (exp(b0 + b1*mean(x1)
+ b2*mean(x2) + b3*mean(x3) + b4*mean(x4))/ (1+exp(b0 + b1*mean(x1)
+ b2*mean(x2) + b3*mean(x3) + b4*mean(x4))))
}'

newlist <- list(num=178,
     y=c(0, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0,
         0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 0, 0, 0,
         0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0, 0,
         0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,
         0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 0,
         0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,
         0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,
         0, 1, 0, 0, 0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 1, 1, 0, 0, 0,
         0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0),
     x1=c(1, 1, 0, 0, 0, 0, 0, 1, 1, 0, 0, 1, 0, 1, 1, 1, 0, 1, 0, 1,
          0, 0, 0, 1, 1, 1, 0, 1, 1, 0, 0, 1, 1, 1, 1, 1, 1, 1, 1, 1,
          1, 1, 0, 1, 0, 1, 1, 0, 1, 0, 0, 1, 1, 1, 1, 0, 1, 0, 1, 1,
          0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 1, 1, 1, 1, 1, 0, 1, 1, 1, 1,
          0, 1, 0, 1, 1, 1, 0, 1, 1, 0, 0, 1, 0, 1, 0, 1, 0, 0, 1, 0,
          1, 1, 1, 1, 1, 1, 0, 1, 1, 1, 0, 1, 0, 0, 0, 0, 0, 0, 0, 1,
```

```
        1, 1, 0, 0, 0, 0, 0, 0, 1, 1, 0, 1, 0, 0, 1, 1, 0, 0, 1, 1,
        0, 0, 0, 0, 0, 1, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 1, 0, 0,
        0, 1, 0, 0, 0, 0, 0, 1, 0, 0, 0, 1, 0, 0, 1, 0, 1, 1),
    x2=c(0, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 1, 1, 1, 1, 0, 0, 1, 1,
        1, 1, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,
        0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,
        0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 1, 0, 0, 0, 1, 0, 0, 0, 0,
        0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0, 0,
        1, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 1, 0, 0, 0,
        0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,
        0, 0, 0, 1, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0, 0, 1, 1, 1, 0, 0,
        1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 1, 0, 0, 0, 0, 0),
    x3=c(1, 1, 0, 1, 0, 0, 1, 1, 0, 0, 0, 0, 0, 0, 0, 0, 1, 1, 0, 0,
        0, 0, 0, 0, 1, 1, 1, 0, 0, 0, 0, 0, 0, 0, 0, 1, 1, 1, 1, 0, 0,
        0, 0, 1, 1, 1, 0, 0, 0, 1, 0, 0, 1, 1, 1, 1, 0, 1, 1, 0, 1,
        1, 1, 0, 1, 1, 0, 0, 0, 1, 1, 1, 0, 1, 1, 0, 0, 0, 0, 0, 0,
        0, 1, 1, 1, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 1, 1, 1,
        0, 1, 0, 0, 0, 0, 1, 1, 0, 0, 0, 0, 1, 0, 0, 1, 1, 1, 0, 1,
        1, 1, 0, 0, 0, 1, 1, 1, 1, 0, 1, 1, 1, 0, 0, 0, 0, 0, 0, 0,
        0, 1, 1, 0, 1, 1, 0, 0, 1, 1, 0, 1, 1, 0, 1, 1, 1, 1, 1, 1,
        1, 0, 0, 0, 0, 0, 0, 1, 1, 1, 1, 0, 0, 0, 0, 0, 0, 0),
    x4=c(1, 0, 1, 1, 1, 1, 1, 0, 1, 1, 0, 1, 1, 1, 1, 1, 0, 0, 0, 0,
        0, 0, 0, 1, 1, 1, 1, 1, 1, 0, 1, 0, 0, 0, 0, 0, 1, 1, 0, 0,
        0, 0, 0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0, 1, 1, 0, 0, 0,
        1, 1, 1, 1, 1, 1, 1, 1, 0, 0, 0, 0, 0, 0, 0, 1, 0, 0, 0, 1,
        1, 1, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 1, 1, 1, 1, 0, 1, 1,
        0, 1, 0, 0, 0, 1, 1, 1, 0, 1, 1, 1, 1, 0, 0, 0, 0, 1, 0, 0,
        0, 0, 1, 1, 1, 1, 1, 0, 0, 1, 0, 0, 1, 1, 1, 0, 1, 1, 1, 1,
        1, 1, 1, 0, 0, 1, 1, 0, 1, 1, 1, 1, 1, 0, 0, 1, 0, 0, 0, 1,
        0, 0, 0, 1, 1, 1, 1, 1, 1, 1, 0, 0, 1, 1, 1, 1, 0, 1),
    x5=c(1, 0, 0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 1, 1, 0, 0, 1, 0,
        0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 1, 1, 1, 1, 0, 0, 0, 0,
        0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0, 0, 1, 0, 0,
        0, 0, 0, 1, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1,
        0, 1, 0, 1, 1, 0, 0, 0, 0, 0, 0, 1, 1, 0, 0, 0, 0, 1, 0, 0,
```

```
          1, 0, 0, 1, 0, 0, 1, 1, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,
          0, 0, 0, 1, 1, 0, 0, 0, 0, 0, 1, 1, 0, 0, 0, 0, 1, 1, 1, 0,
          0, 0, 1, 1, 1, 0, 1, 0, 0, 0, 0, 1, 0, 0, 1, 0, 1, 1, 0, 0,
          0, 0, 0, 0, 1, 1, 1, 0, 0, 0, 0, 0, 0, 1, 1, 1, 1, 0))


jags <- jags.model(textConnection(mod.mult), data = newlist,
n.chains = 3, n.adapt = 20000)
results=coda.samples(jags, variable.names=c('b0', 'b1', 'b2',
'b3', 'b4', 'b5', 'rr1', 'rr2', 'rr3', 'rr4', 'rr5'),
n.iter=30000, thin=1)
summary(results)
```

.5 Appendix V: Veterinarian questionnaire

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New Zealand
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Facsimile: +64 (6) 350 5716
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Study of Leptospirosis in veterinarians with exposure to farm animals

Participant Questionnaire

The research team appreciates your involvement in this study of leptospirosis and is committed to privacy of all personal information.

The information from this questionnaire will help us to assess the risk of veterinarians contracting leptospirosis on farms and to develop control strategies.

Personal information included in the questionnaire will be treated in confidence and will not be published or disclosed to any third parties (for example your employer) by the research team in a manner that would allow identification of participants. Results of tests will be copied to your GP only if you have given consent.

Date of questionnaire completion ____/____/2012

1. Participant identification

Name & Surname	
Did you fill in a consent and confidentiality form?	Yes ☐ No ☐ Please get both forms filled in now
Postal Address	
Email	
Contact phone number	
Communication of results	Postal address ☐ Email ☐
Age	
Gender	Male ☐ Female ☐

2. Exposure at work

When did you graduate?	In year _____
For how long have you been working as a veterinarian?	_____ years
Do you work full time?	Full time ☐ Part-time ☐
During your entire working life, please estimate how long you spent with these species (as % of total time in practice)	Dairy _____% Beef _____% Sheep _____% Deer _____% Dog/cat _____% Other _____% No contact _____ %
In the past 18 months, how much time did you work with these species (as % of total time in practice)	Dairy _____% Beef _____% Sheep _____%

	Deer _____% Dog/cat _____% Other _____% No contact _____ %
What percentage of your client farmers vaccinate against Leptospirosis?	Dairy _____% Beef _____% Sheep _____% Deer _____% Other (specify) _____%
Of those client farmers who vaccinate, how many are vaccinated by the farmer himself?	Dairy _____% Beef _____% Sheep _____% Deer _____% Other (specify) _____%
Do you believe Leptospirosis presents a serious health risk at your work?	Definitely ☐ Probably ☐ Maybe ☐ No ☐
Do you wear glasses for protection when working with farm animals	Regularly ☐ Often ☐ Sometimes ☐ Never ☐ I always wear spectacles ☐
Do you band-aid cuts/scratches on your hands	Always ☐ Usually ☐ Sometimes ☐ Never ☐
Do you wash your hands immediately after every contact you have with livestock?	Always ☐ Regularly ☐ Sometimes ☐ Never ☐ N/A ☐
If you wash hands after animal contact, what are the most common drying facilities?	Paper towels ☐ Fabric towel ☐ Air ☐ None ☐ Other (specify):_____
If you wash hands after animal contact, do you use a disinfectant?	Always ☐ Often ☐ Sometimes ☐ Never ☐
Do you routinely wear latex gloves when at risk of urine contamination (e.g. pregnancy testing, calving cows/lambing ewes?	Regularly ☐ Often ☐ Sometimes ☐ Never ☐ If Never, why? _____

3. Exposure outside the veterinary practice

Do you own any livestock or pigs and handle these animals yourself.	No ☐ If yes, mention species _____
Do you have pets in/around the house?	No ☐ If yes, mention species _____
If yes, have they been vaccinated regularly against leptospirosis?	Yes ☐ Species _____ No ☐ Don't know ☐ Yes ☐ Species _____ No ☐ Don't know ☐ Yes ☐ Species _____ No ☐ Don't know ☐

Home slaughter

Have you been involved in home slaughter in the past 18 months? (for human consumption or dog tucker)?

NO ☐ YES ☐ (if yes, species)_____

Have you hunted or been in contact with wild animals in the last 18 months?

NO ☐ YES ☐ (if yes, species)_____

Outdoor exposures

In the last 18 months, have you done outdoor activities where you were exposed to fresh water?

NO ☐ YES ☐

If YES:
- ☐ Camping beside lakes/rivers
- ☐ Water sports in lakes/rivers
- ☐ Fresh water fishing
- ☐ Tramping
- ☐ Other (specify)_____

4. Previous illness

Have you been diagnosed with leptospirosis?

YES ☐ Complete **Lepto Table 4.1 and 4.2**

NO / Don't know ☐ Go to **Flu-like symptoms Table 4.3**

4.1 If you were ill with leptospirosis, please enter details in the table below:

How many times did you have an illness diagnosed as leptospirosis?		
Do you know the serovar(s) and/or titre(s)? Please include information from all tests if tested multiple times, starting with the most recent	Serovar(s)_____ Titre(s):_____	No ☐

4.2 Please enter details about your last episode of leptospirosis in the table below:

Approximate month/year that you were diagnosed with	_____ / _____

leptospirosis:		
How was it diagnosed?	Self-diagnosed ☐ GP ☐ Laboratory test ☐	
If leptospirosis was confirmed by laboratory test, what test was used?	Culture or PCR ☐ Serology ☐ Other (specify): _____	
Do you know or suspect the source of infection?	Yes: _____	No ☐
How many days were you off-work or seriously ill?	_____ days	
Were you hospitalised?	Yes ☐ Number of days_____ No ☐	
Which of the following symptoms did you experience?	☐ Fever ☐ Headache ☐ Sore muscles ☐ Sore eyes ☐ Sweating ☐ Severe debility ☐ Photophobia ☐ Other: _____	
Was it treated?	Yes ☐ No ☐ Don't remember ☐	
If treated, what kind of treatment did you receive?	Antibiotic treatment ☐ how many days: _____ Other treatment ☐ Please detail_____	
Did you receive ACC compensation?	Yes ☐ No ☐ Don't remember ☐	

4.3 Flu-like symptoms

In general if you have flu-like symptoms* would you consult a doctor?	Always ☐ Mostly ☐ Rarely ☐ Never ☐	
Have you had any flu-like symptoms* in the last 18 months?	Yes ☐ Approx month/year _____ / _____	No ☐ **go to section 5**

***** e.g. fever, headache, sore muscles or bones, sore eyes, sweating, severe general debility

4.4 Other Illness

Have you been off work due to this illness in the last 18 months?	Yes ☐ # days_____	No ☐

When you were ill, did you ask for professional help?	GP ☐ Nurse ☐ Other_____	No ☐
Were any blood tests done or samples collected?	Yes ☐ No ☐ Don't remember ☐	
Was a diagnosis made?	Yes ☐ Diagnosis:_____	No ☐ Don't remember ☐
Which of the following symptoms did you have?	☐ Fever ☐ Headache ☐ Sore muscles ☐ Sore eyes ☐ Sweating ☐ Severe debility ☐ Photophobia ☐ Other: _____	
Was it treated?	Yes ☐ No ☐ Don't remember ☐	
If treated, what kind of treatment did you receive?	Antibiotic treatment ☐ how many days: _____ Other treatment ☐ Please detail_____	

5. Your opinions

Do you discuss Leptospirosis as a public health issue with your clients?	Regularly ☐ Often ☐ Sometimes ☐ Never ☐
Do you discuss the human health risk of leptospirosis with your clients? If yes, please state with how many clients (%). [for example: 40% Dairy and 10% Sheep clients]; leave blank if your clients do not own the species	Dairy_____(%) Sheep_____(%) Beef_____(%) Deer_____(%) Other (specify)_____(%) Comment: _____
Do you think farmers should be encouraged to vaccinate their livestock against leptospirosis? If yes, for what purpose? Please insert letter(s): (A) Protecting humans (B) Preventing clinical disease in animals (C) Increasing growth rates in animals (D) Preventing reproductive loss in animals	Dairy Yes ☐/ No ☐ Purpose(s)_____ Beef Yes ☐/ No ☐ Purpose(s)_____ Sheep Yes ☐/ No ☐ Purpose(s)_____ Deer Yes ☐/ No ☐ Purpose(s)_____ Other species _____ Purpose(s)_____ Other species _____ Purpose(s)_____

In your opinion, what information is required (and not available) about vaccinating animals against leptospirosis?	
What do you think is the probability (%) that you are leptospirosis seropositive?	

THANK YOU!

This is the end of the questionnaire. The research team appreciates your involvement in this study of leptospirosis and is committed to privacy of all personal information..

Your blood serum will be tested for antibodies to *Leptospira* serovars Ballum, Pomona and Hardjo and you will be notified of the result within approximately two months.

Results of the entire study will be made available through NZVA and publications.

.6 Appendix VI: Burden and cost Code

```
# Number of incident cases
n <- 1e+05
mw.ca <- numeric(n)
par.mw <- numeric(n)
far.ca <- numeric(n)
par.far <- numeric(n)
vet.ca <- numeric(n)
par.vet <- numeric(n)
noatrisk.ca <- numeric(n)
tot.ca <- numeric(n)
atrisk.ca <- numeric(n)

mw.pop <- 16224
vet.pop <- 1989
dairy.pop <- 26577
beef.pop <- 3924
deer.pop <- 387
sheep.pop <- 9297
mixed.pop <- 30276
far.pop <- dairy.pop + beef.pop + deer.pop + sheep.pop +
    mixed.pop
farm.pop.novac <- (dairy.pop * (1 - 0.9 * 0.82)) +
    (beef.pop * (1 - 0.21 * 0.82)) + (deer.pop *
    (1 - 0.08 * 0.82)) + (sheep.pop * (1 - 0.006 *
    0.82)) + (mixed.pop * (1 - 0.006 * 0.82))
noatrisk.pop <- 3670750 - (mw.pop + vet.pop +
    far.pop)

mw.ca.svr <- numeric(n)
mw.ca.mild <- numeric(n)
far.ca.svr <- numeric(n)
far.ca.mild <- numeric(n)
vet.ca.svr <- numeric(n)
```

```
vet.ca.mild <- numeric(n)
noatrisk.ca.svr <- numeric(n)
noatrisk.ca.mild <- numeric(n)
atrisk.ca.svr <- numeric(n)
atrisk.ca.mild <- numeric(n)
tot.ca.svr <- numeric(n)
tot.ca.mild <- numeric(n)
severe <- numeric(n)

library(prevalence)
bmw <- betaExpert(best = 0.02826, lower = , upper = 0.046029419,
    p = 0.95, method = "mode")
bvet <- betaExpert(best = 0.008065 * 2/3, lower = ,
    upper = 0.020683432 * 2/3, p = 0.95, method = "mode")
bfar <- betaExpert(best = 0.02067 * 2/3, lower = ,
    upper = 0.0415024301 * 2/3, p = 0.95, method = "mode")

svr <- betaExpert(best = 0.1364, lower = , upper = 0.3333,
    p = 0.95, method = "mode")
chro <- betaExpert(best = 0.302, lower = , upper = 0.362,
    p = 0.95, method = "mode")

I.mild.atrisk <- numeric(n)
I.severe.atrisk <- numeric(n)
I.mild.noatrisk <- numeric(n)
I.severe.noatrisk <- numeric(n)
I.mild.total <- numeric(n)
I.severe.total <- numeric(n)
I.chronic <- numeric(n)

for (i in 1:n) {
    par.mw[i] <- rbeta(1, bmw[[1]], bmw[[2]])
    par.vet[i] <- rbeta(1, bvet[[1]], bvet[[2]])
    par.far[i] <- rbeta(1, bfar[[1]], bfar[[2]])
    mw.ca[i] <- par.mw[i] * mw.pop
```

```
    far.ca[i] <- par.far[i] * farm.pop.novac
    vet.ca[i] <- par.vet[i] * vet.pop
    atrisk.ca[i] <- mw.ca[i] + far.ca[i] + vet.ca[i]
    noatrisk.ca[i] <- 0.181 * atrisk.ca[i]/0.819
    tot.ca[i] <- mw.ca[i] + far.ca[i] + vet.ca[i] +
        noatrisk.ca[i]
    severe[i] <- rbeta(1, svr[[1]], svr[[2]])
    mw.ca.svr[i] <- mw.ca[i] * severe[i]
    mw.ca.mild[i] <- mw.ca[i] - mw.ca.svr[i]
    far.ca.svr[i] <- far.ca[i] * severe[i]
    far.ca.mild[i] <- far.ca[i] - far.ca.svr[i]
    vet.ca.svr[i] <- vet.ca[i] * severe[i]
    vet.ca.mild[i] <- vet.ca[i] - vet.ca.svr[i]
    atrisk.ca.svr[i] <- mw.ca.svr[i] + far.ca.svr[i] +
        vet.ca.svr[i]
    atrisk.ca.mild[i] <- mw.ca.mild[i] + far.ca.mild[i] +
        vet.ca.mild[i]
    noatrisk.ca.svr[i] <- noatrisk.ca[i] * severe[i]
    noatrisk.ca.mild[i] <- noatrisk.ca[i] - noatrisk.ca.svr[i]
    tot.ca.svr[i] <- tot.ca[i] * severe[i]
    tot.ca.mild[i] <- tot.ca[i] - tot.ca.svr[i]
    I.severe.atrisk[i] <- mw.ca.svr[i] + far.ca.svr[i] +
        vet.ca.svr[i]
    I.mild.atrisk[i] <- mw.ca.mild[i] + far.ca.mild[i] +
        vet.ca.mild[i]
    I.severe.noatrisk[i] <- noatrisk.ca.svr[i]
    I.mild.noatrisk[i] <- noatrisk.ca.mild[i]
    I.severe.total[i] <- mw.ca.svr[i] + far.ca.svr[i] +
        vet.ca.svr[i] + noatrisk.ca.svr[i]
    I.mild.total[i] <- mw.ca.mild[i] + far.ca.mild[i] +
        vet.ca.mild[i] + noatrisk.ca.mild[i]
}

# DALYs estimation
DW.mild <- numeric(n)
```

```
DW.severe <- numeric(n)
DW.postacute <- numeric(n)

YLD.mild.atrisk <- numeric(n)
YLD.severe.atrisk <- numeric(n)
YLD.mild.noatrisk <- numeric(n)
YLD.severe.noatrisk <- numeric(n)
YLD.mild.total <- numeric(n)
YLD.severe.total <- numeric(n)
D.severe <- numeric(n)
D.mild <- numeric(n)
D.postacute.geo <- numeric(n)

for (i in 1:n) {
    D.severe[i] <- rpois(n = 1, lambda = 16)/365
    D.mild[i] <- rpois(n = 1, lambda = 4)/365
    D.postacute.geo[i] <- (rgeom(1, 0.065) + 0.1)/12
    I.chronic[i] <- rbeta(1, chro[[1]], chro[[2]])
    DW.mild[i] <- runif(1, 0.033, 0.081)
    DW.severe[i] <- runif(1, 0.139, 0.298)
    DW.postacute[i] <- runif(1, 0.17, 0.355)
    YLD.mild.atrisk[i] <- I.mild.atrisk[i] * DW.mild[i] *
        D.mild[i]
    YLD.severe.atrisk[i] <- (I.severe.atrisk[i] *
        DW.severe[i] * D.severe[i]) + (I.severe.atrisk[i] *
        I.chronic[i] * DW.postacute[i] * D.postacute.geo[i])
    YLD.mild.noatrisk[i] <- I.mild.noatrisk[i] *
        DW.mild[i] * D.mild[i]
    YLD.severe.noatrisk[i] <- (I.severe.noatrisk[i] *
        DW.severe[i] * D.severe[i]) + (I.severe.noatrisk[i] *
        I.chronic[i] * DW.postacute[i] * D.postacute.geo[i])
    YLD.mild.total[i] <- I.mild.total[i] * DW.mild[i] *
        D.mild[i]
    YLD.severe.total[i] <- (I.severe.total[i] *
        DW.severe[i] * D.severe[i]) + (I.severe.total[i] *
```

```
        I.chronic[i] * DW.postacute[i] * D.postacute.geo[i])
}

DALY.atrisk <- numeric(n)
DALY.noatrisk <- numeric(n)
DALY.total <- numeric(n)

for (i in 1:n) {
    DALY.atrisk[i] <- YLL[i] + YLD.mild.atrisk[i] +
        YLD.severe.atrisk[i]
    DALY.noatrisk[i] <- YLL[i] + YLD.mild.noatrisk[i] +
        YLD.severe.noatrisk[i]
    DALY.total[i] <- YLL[i] + YLD.mild.total[i] +
        YLD.severe.total[i]
}

# Cost of work absence and treatment
offwork.cost <- numeric(n)
offwork.cost2 <- numeric(n)
absence.cost <- numeric(n)
absence.cost2 <- numeric(n)
absence.cost3 <- numeric(n)
acc.cost.claim <- numeric(n)
acc.cost <- numeric(n)
tot.cost.acc.abs <- numeric(n)
tot.cost.acc.abs.per100th <- numeric(n)
tot.cost.acc.abs2 <- numeric(n)
tot.cost.acc.abs3 <- numeric(n)

for (i in 1:n) {
    offwork.cost[i] <- 183
    offwork.cost2[i] <- 616
    absence.cost[i] <- offwork.cost[i] * ((D.mild[i] *
        365 * I.mild.total[i]) + (D.severe[i] *
        365 * I.severe.total[i]) + (2/12 * 365 *
```

```
        I.severe.total[i] * I.chronic[i]))
    absence.cost2[i] <- offwork.cost[i] * ((D.mild[i] *
        365 * I.mild.total[i]) + (D.severe[i] *
        365 * I.severe.total[i]) + (D.postacute.geo[i] *
        365 * I.severe.total[i] * I.chronic[i]))
    absence.cost3[i] <- offwork.cost2[i] * ((D.mild[i] *
        365 * I.mild.total[i]) + (D.severe[i] *
        365 * I.severe.total[i]) + (2/12 * 365 *
        I.severe.total[i] * I.chronic[i]))
    acc.cost.claim[i] <- rnorm(n = 1, mean = 10950,
        sd = 6958.594)
    acc.cost[i] <- (acc.cost.claim[i] * I.severe.total[i] *
        1/3) + (acc.cost.claim[i] * 0.5 * I.severe.total[i] *
        1/3) + (acc.cost.claim[i] * 0.25 * I.severe.total[i] *
        1/3) + (acc.cost.claim[i] * 0.01 * I.mild.total[i] *
        1/2)
    tot.cost.acc.abs[i] <- acc.cost[i] + absence.cost[i]
    tot.cost.acc.abs.per100th[i] <- (acc.cost[i] +
        absence.cost[i]) * 1e+05/4242048
    # post-acute
    tot.cost.acc.abs2[i] <- acc.cost[i] + absence.cost2[i]
    # 616 estimate of day off work
    tot.cost.acc.abs3[i] <- acc.cost[i] + absence.cost3[i]
}

# Production loss SHEEP parameters
pop.s <- 18890000
kg.lost.s <- numeric(n)
kg.value.s <- numeric(n)
far.exp.s <- numeric(n)
sheep.exp <- numeric(n)
farm.vac.s <- numeric(n)
far.aff.s <- numeric(n)
tail.r <- numeric(n)
lambs.aff <- numeric(n)
```

```
kg.c.s <- numeric(n)
rep.rate.s <- numeric(n)
dressing.p.s <- numeric(n)

f.exp.pom <- numeric(n)
s.exp.pom <- numeric(n)
f.aff.pom <- numeric(n)
s.aff.pom <- numeric(n)
ab.exp <- numeric(n)
fet.lost.s <- numeric(n)
profit.ewe <- numeric(n)
fetloss.c.s <- numeric(n)
tot.loss.s <- numeric(n)

library(prevalence)
farms.exposed.s <- betaExpert(best = 0.967, lower = 0.908,
    upper = , p = 0.95, method = "mode")
sheep.exposed <- betaExpert(best = 0.543, lower = 0.445,
    upper = , p = 0.95, method = "mode")
farms.affected.s <- betaExpert(best = 0.125, lower = ,
    upper = 0.471, p = 0.95, method = "mode")
vac.sheep <- betaExpert(best = 0.006, lower = ,
    upper = 0.018, p = 0.95, method = "mode")

flocks.exp.pom <- betaExpert(best = 0.761, lower = 0.664,
    upper = , p = 0.95, method = "mode")
sheep.exp.pom <- betaExpert(best = 0.087, lower = ,
    upper = 0.114, p = 0.95, method = "mode")
farm.sheep.aff.pom <- betaExpert(best = 0.01,
    lower = , upper = 0.05, p = 0.95, method = "mode")
sheep.aff.pom <- betaExpert(best = 0.75, lower = 0.5,
    upper = , p = 0.95, method = "mode")
replacement.rate.s <- betaExpert(best = 0.3, lower = ,
    upper = 0.4, p = 0.95, method = "mode")
dressing.percent.s <- betaExpert(best = 0.42,
```

```
    lower = , upper = 0.48, p = 0.95, method = "mode")

# 3. BEEF parameters
pop.b <- 970000
far.exp.b <- numeric(n)
an.exp.b <- numeric(n)
farm.vac.b <- numeric(n)
exp.aff.b <- numeric(n)
kg.value.b <- numeric(n)
steers.aff <- numeric(n)
kg.lost.b <- numeric(n)
kg.c.b <- numeric(n)
rep.rate.b <- numeric(n)
wean.rate.b <- numeric(n)
dressing.p.b <- numeric(n)

farms.exposed.beef <- betaExpert(best = 0.985,
    lower = 0.919, upper = , p = 0.95, method = "mode")
beef.exposed <- betaExpert(best = 0.653, lower = 0.548,
    upper = , p = 0.95, method = "mode")
vacc.beef <- betaExpert(best = 0.214, lower = ,
    upper = 0.243, p = 0.95, method = "mode")
beef.exposed.affect <- betaExpert(best = 0.14,
    lower = , upper = 0.51, p = 0.95, method = "mode")
replacement.rate.b <- betaExpert(best = 0.2, lower = ,
    upper = 0.25, p = 0.95, method = "mode")
weaning.rate.b <- betaExpert(best = 0.8, lower = 0.75,
    upper = , p = 0.95, method = "mode")
dressing.percent.b <- betaExpert(best = 0.45,
    lower = , upper = 0.53, p = 0.95, method = "mode")

PAF.abo.b <- numeric(n)
abo.rate.b <- numeric(n)
fet.lost.b <- numeric(n)
rev.calf.b <- numeric(n)
```

```
fetloss.c.b <- numeric(n)
tot.loss.b <- numeric(n)

# 4. DEER parameters
pop.d <- 458100
far.exp.d <- numeric(n)
ani.exp.d <- numeric(n)
farm.vac.d <- numeric(n)
wean.rate.d <- numeric(n)
cal.weaned.d <- numeric(n)
rep.rate.d <- numeric(n)
cal.venison.d <- numeric(n)
far.aff.d <- numeric(n)
grow.aff.d <- numeric(n)
kg.value.d <- numeric(n)
kg.lost.d <- numeric(n)
kg.lost.c.d <- numeric(n)
dressing.p.d <- numeric(n)

# betas
farms.exp.d <- betaExpert(best = 0.811, lower = 0.728,
    upper = , p = 0.95, method = "mode")
animals.exp.d <- betaExpert(best = 0.608, lower = 0.587,
    upper = , p = 0.95, method = "mode")
weaning.rate.d <- betaExpert(best = 0.8, lower = 0.7,
    upper = , p = 0.95, method = "mode")
replacement.rate.d <- betaExpert(best = 0.2, lower = ,
    upper = 0.3, p = 0.95, method = "mode")
farms.affect.d <- betaExpert(best = 1, lower = 0.9,
    upper = , p = 0.95, method = "mode")
vacc.deer <- betaExpert(best = 0.076, lower = ,
    upper = 0.107, p = 0.95, method = "mode")
dressing.percent.d <- betaExpert(best = 0.56,
    lower = , upper = 0.6, p = 0.95, method = "mode")
increase.mort.d <- betaExpert(best = 0.06, lower = ,
```

```
    upper = 0.27, p = 0.95, method = "mode")

incr.mort.d <- numeric(n)
cal.aff.d <- numeric(n)
weaner.val.d <- numeric(n)
mort.c.d <- numeric(n)
tot.loss.d <- numeric(n)

# Total parameters
tot.an.c <- numeric(n)
tot.c <- numeric(n)
tot.an.c2 <- numeric(n)

for (i in 1:n) {
    # 2. SHEEP model SHEEP: growth
    far.exp.s[i] <- rbeta(1, farms.exposed.s[[1]],
        farms.exposed.s[[2]])
    sheep.exp[i] <- rbeta(1, sheep.exposed[[1]],
        sheep.exposed[[2]])
    farm.vac.s[i] <- rbeta(1, vac.sheep[[1]],
        vac.sheep[[2]])
    far.aff.s[i] <- rbeta(1, farms.affected.s[[1]],
        farms.affected.s[[2]])
    tail.r[i] <- 1.28
    dressing.p.s[i] <- rbeta(1, dressing.percent.s[[1]],
        dressing.percent.s[[2]])
    kg.lost.s[i] <- 0.636
    kg.value.s[i] <- rnorm(1, 4.5, 0.5)
    rep.rate.s[i] <- rbeta(1, replacement.rate.s[[1]],
        replacement.rate.s[[2]])
    lambs.aff[i] <- pop.s * tail.r[i] * (1 - rep.rate.s[i]) *
        (1 - farm.vac.s[i]) * far.exp.s[i] * sheep.exp[i] *
        far.aff.s[i]
    kg.c.s[i] <- lambs.aff[i] * kg.lost.s[i] *
        kg.value.s[i] * dressing.p.s[i]
```

```
# SHEEP: foetal loss
f.exp.pom[i] <- rbeta(1, flocks.exp.pom[[1]],
    flocks.exp.pom[[2]])
s.exp.pom[i] <- rbeta(1, sheep.exp.pom[[1]],
    sheep.exp.pom[[2]])
f.aff.pom[i] <- rbeta(1, farm.sheep.aff.pom[[1]],
    farm.sheep.aff.pom[[2]])
s.aff.pom[i] <- 0.75
fet.lost.s[i] <- pop.s * tail.r[i] * (1 -
    farm.vac.s[i]) * f.exp.pom[i] * s.exp.pom[i] *
    f.aff.pom[i] * s.aff.pom[i]
profit.ewe[i] <- rnorm(1, 30, 10)
fetloss.c.s[i] <- fet.lost.s[i] * profit.ewe[i]
tot.loss.s[i] <- kg.c.s[i] + fetloss.c.s[i]

# 3. BEEF model BEEF: growth
far.exp.b[i] <- rbeta(1, farms.exposed.beef[[1]],
    farms.exposed.beef[[2]])
an.exp.b[i] <- rbeta(1, beef.exposed[[1]],
    beef.exposed[[2]])
farm.vac.b[i] <- rbeta(1, vacc.beef[[1]],
    vacc.beef[[2]])
exp.aff.b[i] <- rbeta(1, beef.exposed.affect[[1]],
    beef.exposed.affect[[2]])
dressing.p.b[i] <- rbeta(1, dressing.percent.b[[1]],
    dressing.percent.b[[2]])
kg.lost.b[i] <- rnorm(1, 14, 5)
wean.rate.b[i] <- rbeta(1, weaning.rate.b[[1]],
    weaning.rate.b[[2]])
rep.rate.b[i] <- rbeta(1, replacement.rate.b[[1]],
    replacement.rate.b[[2]])
steers.aff[i] <- pop.b * wean.rate.b[i] *
    (1 - rep.rate.b[i]) * (1 - farm.vac.b[i]) *
    far.exp.b[i] * an.exp.b[i] * exp.aff.b[i]
kg.value.b[i] <- rnorm(1, 4.5, 1.5)
```

```
    kg.c.b[i] <- steers.aff[i] * kg.lost.b[i] *
        kg.value.b[i] * dressing.p.b[i]

    ## BEEF: foetal loss
    PAF.abo.b[i] <- 0.083
    abo.rate.b[i] <- 0.03
    fet.lost.b[i] <- pop.b * abo.rate.b[i] * PAF.abo.b[i]
    rev.calf.b[i] <- rnorm(1, 180, 60)
    fetloss.c.b[i] <- fet.lost.b[i] * rev.calf.b[i]
    tot.loss.b[i] <- kg.c.b[i] + fetloss.c.b[i]

    # 4. DEER model DEER: growth
    far.exp.d[i] <- rbeta(1, farms.exp.d[[1]],
        farms.exp.d[[2]])
    ani.exp.d[i] <- rbeta(1, animals.exp.d[[1]],
        animals.exp.d[[2]])
    wean.rate.d[i] <- rbeta(1, weaning.rate.d[[1]],
        weaning.rate.d[[2]])
    cal.weaned.d[i] <- pop.d * wean.rate.d[i]
    rep.rate.d[i] <- rbeta(1, replacement.rate.d[[1]],
        replacement.rate.d[[2]])
    farm.vac.d[i] <- rbeta(1, vacc.deer[[1]],
        vacc.deer[[2]])

    cal.venison.d[i] <- cal.weaned.d[i] * (1 -
        rep.rate.d[i])
    far.aff.d[i] <- rbeta(1, farms.affect.d[[1]],
        farms.affect.d[[2]])
    grow.aff.d[i] <- far.exp.d[i] * ani.exp.d[i] *
        far.aff.d[i] * cal.venison.d[i] * (1 -
        farm.vac.d[i])
    dressing.p.d[i] <- rbeta(1, dressing.percent.d[[1]],
        dressing.percent.d[[2]])
    kg.lost.d[i] <- 3
    kg.value.d[i] <- rnorm(1, 7.5, 0.5)
```

```
    kg.lost.c.d[i] <- grow.aff.d[i] * kg.lost.d[i] *
        kg.value.d[i] * dressing.p.d[i]

    # DEER: weaning i.e.increased mortality
    incr.mort.d[i] <- rbeta(1, increase.mort.d[[1]],
        increase.mort.d[[2]])
    cal.aff.d[i] <- far.exp.d[i] * cal.weaned.d[i] *
        (1 - farm.vac.d[i]) * ani.exp.d[i] * far.aff.d[i] *
        incr.mort.d[i]
    weaner.val.d[i] <- rnorm(1, 270, 50)
    mort.c.d[i] <- weaner.val.d[i] * cal.aff.d[i]
    tot.loss.d[i] <- kg.lost.c.d[i] + mort.c.d[i]
    tot.an.c[i] <- tot.loss.s[i] + tot.loss.b[i] +
        tot.loss.d[i]
    tot.an.c2[i] <- tot.loss.b[i] + tot.loss.d[i]
}

# Vaccine cost Entire population
pop.dairy <- 5e+06
pop.beef <- 3590000
pop.deer <- 949400
pop.sheep <- 28570000
# Females
pop.b
pop.d
pop.s
pop.da <- 5e+06
vac.cost.bd <- numeric(n)
vac.cost.s <- numeric(n)
# population vaccinated
vac.dairy.cov <- betaExpert(best = 0.9, lower = 0.8,
    upper = , p = 0.95, method = "mode")
vac.beef.cov <- betaExpert(best = 0.214, lower = ,
    upper = 0.243, p = 0.95, method = "mode")
vac.deer.cov <- betaExpert(best = 0.076, lower = ,
```

```
    upper = 0.107, p = 0.95, method = "mode")
vac.sheep.cov <- betaExpert(best = 0.006, lower = ,
    upper = 0.018, p = 0.95, method = "mode")
# Replacement rate
replacement.rate.s
replacement.rate.d
replacement.rate.b
replacement.rate.dairy <- betaExpert(best = 0.22,
    lower = , upper = 0.35, p = 0.95, method = "mode")

# costs
cost.dairy <- numeric(n)
cost.beef <- numeric(n)
cost.deer <- numeric(n)
cost.sheep <- numeric(n)
vac.dairy <- numeric(n)
vac.beef <- numeric(n)
vac.deer <- numeric(n)
vac.sheep <- numeric(n)
rep.rate.da <- numeric(n)
total.vac.cost <- numeric(n)
total.vac.cost.per100th <- numeric(n)
total.vac.cost.per100thp <- numeric(n)
total.vac.cost.dry <- numeric(n)
cost.dairy.booster <- numeric(n)
cost.dairy.calves <- numeric(n)
cost.dairy.total <- numeric(n)
cost.dairy.total.per100th <- numeric(n)
cost.dairy.total.per100thp <- numeric(n)
cost.beef.booster <- numeric(n)
cost.beef.calves <- numeric(n)
cost.beef.total <- numeric(n)
cost.beef.total.per100th <- numeric(n)
cost.beef.total.per100thp <- numeric(n)
cost.deer.booster <- numeric(n)
```

```
cost.deer.calves <- numeric(n)
cost.deer.total <- numeric(n)
cost.deer.total.per100th <- numeric(n)
cost.deer.total.per100thp <- numeric(n)
cost.sheep.booster <- numeric(n)
cost.sheep.lambs <- numeric(n)
cost.sheep.total <- numeric(n)
cost.sheep.total.per100th <- numeric(n)
cost.sheep.total.per100thp <- numeric(n)

for (i in 1:n) {
    vac.cost.bd[i] <- 1.5
    vac.cost.s[i] <- 1.125
    vac.dairy[i] <- 0.9
    vac.beef[i] <- rbeta(1, vac.beef.cov[[1]],
        vac.beef.cov[[2]])
    vac.deer[i] <- rbeta(1, vac.deer.cov[[1]],
        vac.deer.cov[[2]])
    vac.sheep[i] <- rbeta(1, vac.sheep.cov[[1]],
        vac.sheep.cov[[2]])
    rep.rate.da[i] <- rbeta(1, replacement.rate.dairy[[1]],
        replacement.rate.dairy[[2]])

    cost.dairy.booster[i] <- pop.da * (1 - rep.rate.da[i]) *
        vac.dairy[i] * vac.cost.bd[i]
    cost.dairy.calves[i] <- pop.da * rep.rate.da[i] *
        vac.dairy[i] * (vac.cost.bd[i] * 2)
    cost.dairy.total[i] <- cost.dairy.booster[i] +
        cost.dairy.calves[i]
    cost.dairy.total.per100th[i] <- cost.dairy.total[i] *
        1e+05/(pop.da * vac.dairy[i])
    cost.dairy.total.per100thp[i] <- cost.dairy.total[i] *
        1e+05/4242048

    cost.beef.booster[i] <- pop.b * (1 - rep.rate.b[i]) *
```

```
    vac.beef[i] * vac.cost.bd[i]
cost.beef.calves[i] <- pop.b * rep.rate.b[i] *
    vac.beef[i] * (vac.cost.bd[i] * 2)
cost.beef.total[i] <- cost.beef.booster[i] +
    cost.beef.calves[i]
cost.beef.total.per100th[i] <- cost.beef.total[i] *
    1e+05/(pop.b * vac.beef[i])
cost.beef.total.per100thp[i] <- cost.beef.total[i] *
    1e+05/4242048

cost.deer.booster[i] <- pop.d * (1 - rep.rate.d[i]) *
    vac.deer[i] * vac.cost.bd[i]
cost.deer.calves[i] <- pop.d * rep.rate.d[i] *
    vac.deer[i] * (vac.cost.bd[i] * 2)
cost.deer.total[i] <- cost.deer.booster[i] +
    cost.deer.calves[i]
cost.deer.total.per100th[i] <- cost.deer.total[i] *
    1e+05/(pop.d * vac.deer[i])
cost.deer.total.per100thp[i] <- cost.deer.total[i] *
    1e+05/4242048

cost.sheep.booster[i] <- pop.s * (1 - rep.rate.s[i]) *
    vac.sheep[i] * vac.cost.s[i]
cost.sheep.lambs[i] <- pop.s * rep.rate.s[i] *
    vac.sheep[i] * (vac.cost.s[i] * 2)
cost.sheep.total[i] <- cost.sheep.booster[i] +
    cost.sheep.lambs[i]
cost.sheep.total.per100th[i] <- cost.sheep.total[i] *
    1e+05/(pop.s * vac.sheep[i])
cost.sheep.total.per100thp[i] <- cost.sheep.total[i] *
    1e+05/4242048

total.vac.cost[i] <- cost.dairy.booster[i] +
    cost.dairy.calves[i] + cost.beef.booster[i] +
    cost.beef.calves[i] + cost.deer.booster[i] +
```

```
        cost.deer.calves[i] + cost.sheep.booster[i] +
        cost.sheep.lambs[i]
    total.vac.cost.dry[i] <- cost.beef.booster[i] +
        cost.beef.calves[i] + cost.deer.booster[i] +
        cost.deer.calves[i] + cost.sheep.booster[i] +
        cost.sheep.lambs[i]
    total.vac.cost.per100th[i] <- cost.dairy.total.per100th[i] +
        cost.beef.total.per100th[i] + cost.deer.total.per100th[i] +
        cost.sheep.total.per100th[i]
    total.vac.cost.per100thp[i] <- cost.dairy.total.per100thp[i] +
        cost.beef.total.per100thp[i] + cost.deer.total.per100thp[i] +
        cost.sheep.total.per100thp[i]
}

# Total cost
total.cost <- numeric(n)
total.cost.notvac <- numeric(n)
for (i in 1:n) {
    total.cost[i] <- tot.cost.acc.abs[i] + tot.an.c[i] +
        total.vac.cost[i]
    total.cost.notvac[i] <- tot.cost.acc.abs[i] +
        tot.an.c[i]
}

tot.cost.beef <- cost.beef.total + tot.loss.b
tot.cost.sheep <- cost.sheep.total + tot.loss.s
tot.cost.deer <- cost.deer.total + tot.loss.d
```

.7 Appendix VII: Burden and cost assumptions

Species	Parameter	Distribution	mean	SD	mode	95%LL	95%UL	Min	Max	Source
Human	Population abattoir worker	Fix	16224							Anonymous (2013)
	Population farmer	Fix	92766							Anonymous (2013)
	Population veterinarian	Fix	1989							Anonymous (2013)
	Working population	Fix	3760750							Anonymous (2016)
	PAR abattoir worker	Beta			0.0283		0.0461			Dreyfus *et al.* (2014)
	PAR farmer	Beta			0.0137		0.0274			Sanhueza *et al.* (2016)
	PAR veterinarian	Beta			0.0053		0.0140			Sanhueza *et al.* (2015)
	Severe illness	Beta			0.1364		0.333			Dreyfus et al. (2014)
	Post-acute illness (severe cases)	Beta			0.302		0.362			Goris *et al.* (2013)
	Disability weight moderate	Uniform						0.033	0.081	Salomon *et al.* (2012)
	Disability weight severe	Uniform						0.139	0.298	Salomon et al. (2012)
	Disability weight post-acute	Uniform						0.17	0.355	Salomon et al. (2012)
	Duration of mild illness (days)	Poisson	5							Dreyfus et al. (2014)
	Duration of severe illness (days)	Poisson	16							Goris et al. (2013)
	Duration of post-acute illness (months)	Geometric	0.065							Goris et al. (2013)
	Absenteeism cost (NZD)	Fix	183							Statistics New Zealand (2015)
	Treatment cost (NZD)	Uniform						3400	23000	Accident Compensation

							Corporation (ACC)
Sheep	Ewe population	Fix	18890000				Beef+Lamb New Zealand (2015b)
	Lambs per ewe	Fix	1.28				Beef+Lamb New Zealand (2015a)
	Replacement rate	Beta			0.30	0.40	Anne Ridler expert opinion
	Vaccination coverage	Beta			0.006	0.018	Dreyfus *et al.* (2011)
	Herd sero-prevalence	Beta			0.967	0.908	Sanhueza et al. (2016)
	Animal sero-prevalence	Beta			0.543	0.445	Sanhueza et al. (2016)
	Herd sero-prevalence Pomona	Beta			0.761	0.664	Sanhueza et al. (2016)
	Animal sero-prevalence Pomona	Beta			0.087	0.114	Sanhueza et al. (2016)
	Live weight loss (Kg)	Fix	0.663				Vallée *et al.* (2014)
	Dressing %	Beta			0.42	0.48	Nicola Schreurs expert opinion
	Weight loss affected %	Beta			0.125	0.471	Vallée et al. (2014)
	Carcass value (NZ$/Kg)	Normal	4.5	1.5			Nicola Schreurs, expert opinion www.interest.co.nz/rural
	Foetal loss Pomona affected farms	Beta			0.01	0.05	Anne Ridler, expert opinion
	Foetal loss Pomona affected animals	Fix	0.75				Ridler *et al.* (2015)

	Lamb profit value (NZ$/animal)	Normal	30	10			Nicola Schreurs expert opinion www.interest.co.nz/rural
	Vaccination cost	Fix	1.125				Farm services
Beef	Breeding cow population	Fix	970000				Beef+Lamb New Zealand (2015b)
	Mating to weaning percentage	Beta			0.80	0.75	Anne Ridler, expert opinion
	Replacement rate	Beta			0.20	0.25	Anne Ridler, expert opinion
	Vaccination coverage	Beta			0.214	0.243	Heuer (2009) and Dreyfus et al. (2011)
	Herd sero-prevalence	Beta			0.985	0.919	Sanhueza et al. (2016)
	Animal sero-prevalence	Beta			0.653	0.548	Sanhueza et al. (2016)
	Live weight loss (Kg)	Normal	14	5			Vallée et al. (2014)
	Dressing %	Beta			0.45	0.53	Nicola Schreurs, expert opinion
	Weight loss affected %	Beta			0.14	0.51	Vallée et al. (2014)
	Carcass value (NZ$/Kg)	Normal	4.5	1.5			Expert opinion (Nicola); www.interest.co.nz/rural; 6 stock units)
	Foetal loss	Fix	0.03				Heuer (2014)
	Percentage of abortions due to Leptospirosis	Fix	0.083				Sanhueza *et al.* (2013)
	Calf profit value (NZD/animal)	Normal	180	60			Expert opinion (Nicola); www.interest.co.nz/rural; 6

							stock units)
	Vaccination cost	Fix	1.5				Farm services
Deer	Hind population	Fix	458100				Deer Industry NZ (2014)
	Mating to weaning percentage	Beta			0.80	0.70	Peter Wilson, expert opinion
	Replacement rate	Beta			0.20	0.30	Peter Wilson, expert opinion
	Vaccine coverage	Beta			0.076	0.107	Wilson *et al.* (2008) and Dreyfus et al. (2011)
	Herd sero-prevalence	Beta			0.811	0.728	Ayanegui-Alcerreca *et al.* (2010)
	Animal sero-prevalence	Beta			0.608	0.587	Ayanegui-Alcerreca et al. (2010)
	Weight loss affected %	Beta			1	0.90	Peter Wilson, expert opinion
	Live weight loss (Kg)	Fix	3.00				Subharat *et al.* (2012) and Ayanegui-Alcerreca (2006)
	Dressing %	Beta			0.56	0.60	Peter Wilson, expert opinion
	Carcass value (NZ$/Kg)	Normal	7.50	0.50			Peter Wilson, expert opinion; www.interest.co.nz/rural
	Reduced weaning rate	Beta			0.06	0.27	Subharat *et al.* (2011)
	Weaning rate loss affected %	Beta			1	0.90	Peter Wilson, expert opinion
	Weaner profit value (NZ$/animal)	Normal	270	50			Peter Wilson, expert opinion
	Vaccination cost	Fix	1.5				Farm services

.8 Appendix VIII: Summary of trials for meta-analysis

Author and Year	Design	Vaccine	Species	Age month	Challenge	Serovar challenge	Vaccine to challenge	Test	Challenge to testing	Vaccine shedding	Vaccine total	Control shedding	Control Total	Efficacy
Cortese *et al.*, 2014[1] **One vaccine dose**	Controlled trial	Monovalent	Bovine	0.8	Artificial	Hardjo	5.2months	Culture	1-35days	0	21	10	11	100.0%
Cortese *et al.*, 2014 Two vaccine doses	Controlled trial	Monovalent	Bovine	0.8	Artificial	Hardjo	0.72months	Culture	1-35days	0	20	10	11	100.0%
Plunkett *et al.*, 2013	Field trial	Monovalent	Bovine	Mixed	Natural	Hardjo	NA	FA	NA	3	24	3	16	33.3%
Zimmerman *et al.*, 2013	Controlled trial	Polyvalent	Bovine	0.93	Artificial	Hardjo	12months	FA	7-55days	1	18	12	18	91.7%
Zimmerman *et al.*, 2013	Controlled trial	Polyvalent	Bovine	0.93	Artificial	Hardjo	12months	Culture	7-55days	4	18	18	18	77.8%
Rinehart, 2012 vaccine a	Controlled trial	Polyvalent	Bovine	6	Artificial	Hardjo	3.5months	Culture	1-8weeks	0	21	11	11	100.0%
Subharat *et al.*, 2012 Farm1[2]	Field trial	Polyvalent	Deer	3	Natural	Hardjo	2months	Culture	NA	0	11	1	9	100.0%
Subharat *et al.*, 2012 Farm1	Field trial	Polyvalent	Deer	3	Natural	Hardjo	2months	PCR	NA	0	11	5	9	100.0%
Subharat *et al.*, 2012 Farm4	Field trial	Polyvalent	Deer	3	Natural	Hardjo	2months	Culture	NA	0	25	0	25	NA
Subharat *et al.*, 2012 Farm4	Field trial	Polyvalent	Deer	3	Natural	Hardjo	2months	PCR	NA	0	25	3	25	100.0%
Zuerner *et al.*, 2011[3]	Controlled trial	Monovalent	Bovine	10	Artificial	Hardjo	12months	Culture	1-6weeks	0	8	7	7	100.0%
Zuerner *et al.*, 2011	Controlled trial	Monovalent	Bovine	10	Artificial	Hardjo	12months	FA	1-6weeks	1	8	7	7	87.5%
Zuerner *et al.*, 2011	Controlled trial	Monovalent	Bovine	10	Artificial	Hardjo	12months	PCR	1-6weeks	6	8	7	7	25.0%
Ellis *et al.*, 2000 challenge 6 months	Controlled trial	Monovalent	Bovine	12	Artificial	Hardjo	6months	PCR	1month	0	8	6	8	100.0%
Ellis *et al.*, 2000 challenge 12 months control A	Controlled trial	Monovalent	Bovine	12	Artificial	Hardjo	12months	PCR	1month	1	8	8	8	87.5%
Bolin *et al.*, 1989b[1] **One vaccine dose**	Controlled trial	Polyvalent	Bovine	24	Artificial	Hardjo	6.5months	FA	0.7 to calving	6	7	5	5	14.3%
Bolin *et al.*, 1989b[1] **One vaccine dose**	Controlled trial	Polyvalent	Bovine	24	Artificial	Hardjo	6.5months	Culture	0.7 to calving	0	7	5	5	100.0%
Bolin *et al.*, 1989b[1] **Two vaccine doses**	Controlled trial	Polyvalent	Bovine	24	Artificial	Hardjo	6.5months	FA	0.7 to calving	7	8	5	5	12.5%
Bolin *et al.*, 1989b[1] **Two vaccine doses**	Controlled trial	Polyvalent	Bovine	24	Artificial	Hardjo	6.5months	Culture	0.7 to calving	1	8	5	5	87.5%
Broughton *et al.*, 1984a Calves	Field trial	Polyvalent	Bovine	3.5	Natural	Hardjo	8.8months	Culture	1-52weeks	0	9	6	10	100.0%
Broughton *et al.*, 1984b Heifers during outbreak	Field trial	Polyvalent	Bovine	10	Natural	Hardjo	1month	Culture	4-56weeks	2	8	9	10	72.2%
Allen *et al.*, 1982[4] **18 weeks post vacc**	Field trial	Polyvalent	Bovine	9.5	Natural	Hardjo	1.4months	DFM	4.2	3	39	17	43	80.5%
Allen *et al.*, 1982 18 weeks post vacc	Field trial	Polyvalent	Bovine	9.5	Natural	Hardjo	1.4months	Culture	4.2	4	39	15	43	70.6%
Allen *et al.*, 1982 22 weeks post vacc	Field trial	Polyvalent	Bovine	9.5	Natural	Hardjo	1.4months	DFM	5.1	5	39	21	41	75.0%
Allen *et al.*, 1982 22 weeks post vacc	Field trial	Polyvalent	Bovine	9.5	Natural	Hardjo	1.4months	Culture	5.1	0	39	10	41	95.0%
Mackintosh *et al.*, 1980	Field trial	Polyvalent	Bovine	10	Natural	Hardjo	0.93months	Culture	0.93	2	8	9	10	72.2%

[1] Results of vaccinated animals in the two vaccine doses categories were combined for the analysis
[2] Streptomycin treated vaccinated versus streptomycin treated unvaccinated animals
[3] Result for vaccine monovalent 1
[4] Single composite effect size estimated for culture and DFM